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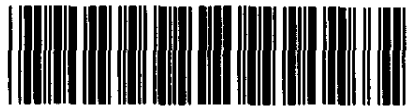

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3:00-CV-02369 NANOGEN INC V. MONTGOMERY

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CMP.

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NANOGEN, INC.

UNITED STATES DISTRICT COURT

FOR THE SOUTHERN DISTRICT OF CALIFORNIA

NANOGEN, INC., a Delaware Corporation,

Plaintiff,

v.

DONALD D. MONTGOMERY, an Individual, and COMBIMATRIX CORP., a Delaware Corporation, and a California Corporation,

Defendants.

CASE NO. 00CV2369 JM RBB

COMPLAINT FOR:

1) Correction of Inventorship of U.S. Patent No. 6,093,302 (35 U.S.C. § 256);

2) Declaratory Ruling of Applicants, Assignee and Inventors

3) and 4) Misappropriation Of Trade Secrets (Cal. Civil Code § 3426.1);

5) and 6) Breach of Written Contract;

7) Inducing Breach Of Written Contract;

8) Breach Of Implied Covenant Of Good Faith And Fair Dealing;

9) Intentional Interference With Prospective Economic Advantage;

10) Unfair Competition (Common Law and Cal. Bus. & Prof. Code §17200);

11) Unjust Enrichment;

12) Constructive Trust;

13) Injunctive Relief; and

14) Conversion.

DEMAND FOR JURY TRIAL

Judge: [not yet assigned]

ORIGINAL

Plaintiff NANOGEN, INC., by and through its designated attorneys, complains and alleges as follows:

I. PARTIES

1. Plaintiff NANOGEN, INC. ("Nanogen") is a corporation organized under the laws of the State of Delaware and has its principal place of business at 10398 Pacific Center Court, San Diego, CA 92121.

2. Upon information and belief, Defendant DONALD D. MONTGOMERY ("Dr. Montgomery") is an individual who is a citizen of the State of Washington and resides in the State of Washington in or near Snoqualmie, WA. Dr. Montgomery was employed by Nanogen from about May of 1994 to about August of 1995.

3. Upon information and belief, Defendant COMBIMATRIX CORP. ("CombiMatrix") is a corporation that was incorporated under the laws of the State of California in October of 1995 with the help of Dr. Montgomery. CombiMatrix Corporation was reincorporated in Delaware in September of 2000. The California Corporation was merged into the Delaware Corporation on September 14, 2000. On information and belief, the California Corporation remains listed as active. The Delaware Corporation is currently existing under the laws of the State of Delaware and has a place of business at 34935 SE Douglas St., Suite 110, Snoqualmie, WA 98065.

II. JURISDICTION AND VENUE

4. This Court has jurisdiction over the first claim alleged herein for "Correction of Inventorship of U.S. Patent No. 6,093,302" and the second claim alleged herein under 35 U.S.C. § 256, 28 U.S.C. § 1331, 28 U.S.C. §§ 1338(a) and (b), and 28 U.S.C. §§ 2201 and 2202. Pursuant to 28 U.S.C. § 1367(a), this Court has supplemental jurisdiction in this matter over each of the following claims:

a. Misappropriation Of Trade Secrets (Cal. Civil Code § 3426 *et seq.*) (two claims);

b. Breach of Written Contract (two claims);

c. Inducing Breach Of Written Contract;

d. Breach Of Implied Covenant Of Good Faith And Fair Dealing;

e. Intentional Interference With Prospective Economic Advantage;

f. Unfair Competition (Common Law and Cal. Bus. & Prof. Code §17200 *et seq.*);

g. Unjust Enrichment;

h. Injunctive Relief;

i. Constructive Trust; and

j. Conversion.

5. Venue is proper in this district under 28 U.S.C. § 1391 for at least the reasons that a substantial part of the events giving rise to the claims of this action occurred in this district, and Nanogen is located in this district and CombiMatrix's contacts with California are in this district.

III. FACTUAL BACKGROUND

Dr. Montgomery's Employment at Nanogen

6. Plaintiff Nanogen is a San Diego biotechnology company that researches, develops, manufactures, markets and intends to market technology that utilizes various aspects of microelectronics, microfabrication, chemistry, and molecular biology. Nanogen is currently developing and has developed instruments and consumables for genomics applications, biomedical research, medical diagnostics, drug discovery and forensics.

7. Maintaining the secrecy of information, including Nanogen's Proprietary Information as defined herein in Paragraph 10, related to the research conducted at, and the technology developed by, Nanogen is important to Nanogen's economic survival. Nanogen derives independent economic value from its Proprietary Information not being generally known to the public or others who could obtain economic value therefrom. Nanogen takes reasonable precautions to protect the secrecy of its Proprietary Information, including its research, data, technology and business plans. Among other precautions, Nanogen obtains its employees' written agreement regarding the disclosure and unauthorized use of Nanogen's confidential and proprietary information.

8. In about May 1994, Nanogen hired Dr. Montgomery to work as a Senior Research Scientist. By agreement with Nanogen, Dr. Montgomery began work on about May 23, 1994. Dr. Montgomery's responsibilities at Nanogen included, among other subjects, research in electrochemistry related to the transport of DNA to spatially multiplexed assay locations for research and diagnostic applications, formulation of permeation layer polymers, solutions for DNA transport and other aspects of using electrodes and electric fields to synthesize polymers and move analytes. Dr. Montgomery terminated his employment at Nanogen on or about August 18, 1995.

9. As part of Dr. Montgomery's employment agreement with Nanogen, he entered into a written Proprietary Information and Inventions Agreement ("Proprietary Information Agreement") with Nanogen. The Proprietary Information Agreement was executed by Dr. Montgomery and approved and agreed to by Nanogen on May 5, 1994. Dr. Montgomery's execution of the Proprietary Information Agreement was a material part of Nanogen's consideration for hiring Montgomery. A true and correct copy of the Agreement is attached to this Complaint as Exhibit "A"[1] and incorporated herein by reference.

10. During the course of his employment, Dr. Montgomery had access to and was exposed to confidential and proprietary information such as the Proprietary Information defined in the Proprietary Information Agreement, including but not limited to, trade secrets, scientific data, invention disclosures, patent applications and drafts thereof, know-how, inventions, strategies, marketing studies, financial information and lists of present and potential financial backers and business partners (collectively, "Proprietary Information").

11. Nanogen's Proprietary Information Agreement, and Nanogen company policy well known to Dr. Montgomery, required that he and all Nanogen scientists maintain daily laboratory notebooks detailing all research, experiments and conclusions, and to submit regular progress reports regarding such during their employment at Nanogen. Nanogen's Proprietary Information Agreement, including the Agreement executed by Dr. Montgomery, as well as Nanogen company policy well known to Dr. Montgomery, also obligated Dr. Montgomery and all Nanogen scientists

[1] Because of the confidential and proprietary information disclosed in the Settlement Agreement, it has been separately under seal with the Court.

to disclose to Nanogen all inventions resulting from work performed during their employment, and to assign ownership of all such inventions to Nanogen. Nanogen's Proprietary Information Agreement, including the Agreement executed by Dr. Montgomery, and company policy well known to Dr. Montgomery, further required Dr. Montgomery and all Nanogen employees to maintain the confidentiality of all research performed at Nanogen, and to keep in confidence any and all of Nanogen's trade secrets and other Proprietary Information of which any and all Nanogen employees, including Dr. Montgomery, became aware during their employment. As with any Nanogen employee, the obligations of assignment and confidentiality, among others, continued in force after Dr. Montgomery terminated his employment with Nanogen.

12. Nanogen is informed and believes, and thereon alleges, that Dr. Montgomery has and continues to retain, use and disclose Nanogen's Proprietary Information in violation of his employment agreement, the Proprietary Information Agreement, and company policy well known to Dr. Montgomery.

13. While affiliated with Nanogen, Dr. Montgomery performed experiments, and was aware of experiments performed by other Nanogen employees, relating to structures and solid phase synthesis methods for the preparation of sequences of biological polymers, including nucleic acid sequences and amino acid sequences using electrochemical placement of monomers on a substrate containing an electrode. Moreover, on information and belief, during his employment at Nanogen Dr. Montgomery performed experiments relating to structures and the placing of a synthesis electrode in contact with a buffering or scavenging solution to prevent chemical crosstalk between electrodes due to diffusion of electrochemically generated reagents, and/or in close proximity to a "getter" structure that could both monitor and obviate ion contamination. Before and/or during Dr. Montgomery's affiliation with Nanogen, experiments conducted by other Nanogen employees, alone or together with Dr. Montgomery's experiments, led to certain inventions which constitute the subject matter of some or all of the technology described and claimed in United States Patent Number 6,093,302 (the "'302 Patent"), PCT Application Number WO 98/01221 (the "'221 PCT"), and PCT Application Number WO 99/35688 (the "'688 PCT"),

all of which are incorporated herein by reference. A true and correct copy of the '302 Patent is attached hereto as Exhibit "B".

14. During his employment at Nanogen, Dr. Montgomery was aware of experiments relating to structures and syntheses of biological polymers at an electrode including, but not limited to, experiments performed by or under the direction of Dr. David Wu and Dr. Theo Nikiforov.

15. Nanogen was aware of research performed and inventions conceived by Nanogen employees, including but not limited to Dr. Wu and Dr. Nikiforov (collectively, the "Nanogen Inventors") and/or Dr. Montgomery constituting or leading to the technology disclosed and claimed in the '302 Patent, the '221 PCT and the '688 PCT. Nanogen had elected to protect any such inventions as trade secrets. At all times, Nanogen was the owner of these trade secrets. Nanogen did not become aware that Dr. Montgomery, for himself or on behalf of CombiMatrix, and CombiMatrix had misappropriated, by disclosure, use or acquisition, of any Nanogen technology, trade secrets or other Proprietary Information until after the publication of the '221 PCT and the '688 PCT; and the issuance of the '302 Patent in July, 2000.

16. On information and belief, the technology disclosed and claimed in the '302 Patent, the '221 PCT and the '688 PCT was conceived and developed by the Nanogen Inventors alone or together with Dr. Montgomery before and/or during Dr. Montgomery's employment at Nanogen.

17. Dr. Montgomery was well aware of his duty to disclose and assign any inventions or technology developed during his employment to Nanogen, as evidenced by his signing of the Proprietary Information Agreement and his disclosure of certain inventions to Nanogen while employed at Nanogen.

18. On information and belief, the laboratory notebooks turned over by Dr. Montgomery upon terminating his employment at Nanogen did not contain all of the scientific research performed by Dr. Montgomery during his employment, as required by the Proprietary Information Agreement and by company policy. Nanogen has been denied access to the records of some of the scientific work performed by Dr. Montgomery during his employment at Nanogen, despite Nanogen's rights to disclosure and ownership of all Dr. Montgomery's research and any technology or inventions derived therefrom during such employment.

19. On information and belief, during Dr. Montgomery's employment at Nanogen and continuing to the present date, Dr. Montgomery concealed from Nanogen his contribution to the research performed at Nanogen by Nanogen employees to develop the technology disclosed and claimed in the '302 Patent, the '221 PCT and the '688 PCT.

20. On information and belief, in about 1995 Dr. Montgomery disclosed to Nanogen employees that he was starting a new venture using a form of combinatorial synthesis technology which is proprietary to Nanogen and owned by Nanogen, and that he had already prepared and presented a business plan to potential investors and had obtained $800,000 in funding from an undisclosed venture capitalist.

21. On information and belief, Dr. Montgomery claimed that he had developed scientific data while employed at Nanogen demonstrating that the above-mentioned synthesis technology would work. Nanogen is informed and believes, and thereon alleges, that Dr. Montgomery obtained and/or developed this data while employed by Nanogen and at Nanogen's expense.

22. Because Dr. Montgomery knew and accepted Nanogen's company policies and the terms of the written Proprietary Information Agreement and again promised and was aware of his obligations in the Settlement Agreement (as detailed in the following paragraph), and because it is customary for biotechnology companies and Nanogen to rely on the representations made by scientists at Dr. Montgomery's level relating to the progress of their scientific research, and because Dr. Montgomery maintained possession of and control over at least some of his laboratory notebooks and other documents containing his scientific research, the facts as set forth herein relating to Dr. Montgomery's research were particularly within Dr. Montgomery's knowledge.

Dr. Montgomery's Activities After Leaving Nanogen

23. Following Dr. Montgomery's voluntary resignation from Nanogen on about August 18, 1995, Dr. Montgomery filed suit in November 1995 against Nanogen for 1) Violation of California Labor Code Section 970; 2) Fraudulent Inducement to Accept Employment; and 3) Constructive Termination in Violation of Public Policy. Montgomery v. Nanogen, Inc., No. 69421 (Cal. Sup. Ct., San Diego 1995). On about February 26, 1996, Dr. Montgomery voluntarily

entered into a Settlement and General Release Agreement ("Settlement Agreement") with Nanogen to settle Dr. Montgomery's lawsuit. In the Settlement Agreement, Dr. Montgomery again covenanted that he would not compete unfairly with Nanogen and would not misappropriate any Nanogen trade secrets. A true and correct copy of the Settlement Agreement is attached hereto as Exhibit "C"[2] and is incorporated herein by reference.

24. On information and belief, Dr. Montgomery actively explored the possibility of commercializing certain inventions made at Nanogen by Nanogen employees, alone or together with Dr. Montgomery for his own benefit. Sometime in about October of 1995, Dr. Montgomery founded and incorporated CombiMatrix in the State of California to directly compete with Nanogen using Nanogen's Proprietary Information, including but not limited to inventions made, conceived, reduced to practice or developed by Nanogen employees and/or by Dr. Montgomery while working at Nanogen.

25. Dr. Montgomery filed a PCT application on July 3, 1997, published on January 15, 1998 as the '221 PCT, claiming priority to a United States Patent Application Serial No. 021,002 filed July 5, 1996, which disclosed and claimed technology developed at Nanogen relating to structures and synthesis methods, using electrochemical placement of monomers or nucleic acids at specific locations on a substrate containing at least one electrode. Dr. Montgomery filed Patent Application No. 09/003,075 with the United States Patent and Trademark Office ("PTO") on January 5, 1998 which ultimately issued on July 25, 2000 as the '302 Patent. Dr. Montgomery filed another PCT application on January 5, 1999, published on July 15, 1999 as the '688 PCT, claiming a priority date of January 5, 1998. Both the '302 Patent and the '688 PCT disclosed and claimed technology developed at Nanogen relating to structures and synthesis methods using electrochemical placement of monomers or nucleic acids at specific locations on a substrate containing at least one electrode in contact with a scavenging solution or proximate to a getter

[2] Because of the confidential and proprietary information disclosed in the Settlement Agreement, it has been filed separately under seal with the Court. Nanogen has concurrently filed *Ex Parte* request for the Court to accept for filing Exhibits A and C under seal.

electrode. Because of the above uses and disclosures, Dr. Montgomery breached his Settlement Agreement with Nanogen.

26. According to the information on the faces of the '302 Patent, the '221 PCT and the '688 PCT, all alleged rights in Dr. Montgomery's patents and applications were assigned to CombiMatrix Corporation. On the '302 Patent, CombiMatrix is listed as the Assignee. On each of the '221 PCT and '688 PCT, CombiMatrix is listed as the Applicant. On information and belief, Dr. Montgomery was a founding stockholder and the Vice President of Research and Development of CombiMatrix at the time the alleged rights in the '302 Patent, the '221 PCT and '688 PCT were granted to CombiMatrix by Dr. Montgomery.

27. On information and belief, all of the claims of the '302 Patent, the '221 PCT and the '688 PCT were conceived at Nanogen by Nanogen employees, all of whom were obligated to assign any and all inventions to Nanogen. Consequently, Nanogen is the rightful owner of the '302 Patent, the '221 PCT and the '688 PCT, and any and all related domestic and foreign patents and patent applications.

IV. CAUSES OF ACTION

FIRST CAUSE OF ACTION

(Correction of Inventorship of U.S. Patent No. 6,093,302 under 35 U.S.C. § 256)

28. Nanogen hereby refers to and incorporates by reference paragraphs 1 through 27, inclusive, of this Complaint.

29. The inventorship on each of the '302 Patent, the '221 PCT and the '688 PCT is currently incorrect because the names of the Nanogen Inventors were omitted.

30. The Nanogen Inventors never signed nor were asked to sign by anyone any Inventors Oath or Declarations or Assignment documents for the '302 Patent, the '221 PCT or the '688 PCT or any other domestic or foreign applications or issued patents claiming priority therefrom or relating to the technology disclosed therein.

31. The Nanogen Inventors each had a duty to assign their rights in the inventions of the '302 Patent, the '221 PCT and the '688 PCT to Nanogen under individual employment agreements and company policy.

32. At all times relevant herein, the Nanogen Inventors acted without deceptive intent with respect to their omissions as inventors or co-inventors of the '302 Patent, the '221 PCT and the '688 PCT and related domestic and foreign patents and patent applications. Only after issuance of the '302 Patent and publication of the '221 PCT and '688 PCT did Nanogen and the Nanogen Inventors became aware that the Nanogen Inventors should have been named as inventors on the '302 Patent, the '221 PCT and the '688 PCT.

33. On information and belief, the Nanogen Inventors, alone or with Dr. Montgomery, employees conceived the subject matter claimed in the '302 Patent, the '221 PCT and the '688 PCT before or during the period of Dr. Montgomery's employment at Nanogen.

34. Experiments, research and invention disclosures of at least several Nanogen employees including Dr. Wu and Dr. Nikiforov indicate that the Nanogen Inventors are inventors of the subject matter claimed in the '302 Patent, the '221 PCT and the '688 PCT.

35. Pursuant to the provisions of 35 U.S.C. § 256, Nanogen requests the Court to order the correction of the inventorship of '302 Patent and any related United States patents and applications by the Commissioner of Patents by the addition of the Nanogen Inventors as inventors or co-inventors.

SECOND CAUSE OF ACTION

(Declaratory Ruling of Proper Applicants, Assignees and Inventors)

36. Nanogen hereby refers to and incorporates by reference Paragraphs 1 through 35, inclusive, of this Complaint.

37. Nanogen requests that this Court, under 28 U.S.C. § 2201 and 2202, declare that the Nanogen Inventors are the inventors or co-inventors, and that Nanogen is the proper assignee and/or Applicant of each of the '302 Patent, the '221 PCT and the '688 PCT, and any domestic or foreign applications, and any issued domestic or foreign patents claiming priority therefrom or related thereto.

THIRD CAUSE OF ACTION

(Misappropriation Of Trade Secrets and Injunction under the California Uniform Trade Secrets Act, Cal. Civil Code §§ 3426 et seq. (by Defendant Dr. Montgomery))

38. Nanogen hereby refers to and incorporates by reference paragraphs 1 through 37, inclusive, of this Complaint.

39. A confidential relationship existed between Nanogen and Dr. Montgomery based on his employment with Nanogen and his Acknowledgement of Nanogen's company policies as set forth in the Employee Handbook, the Proprietary Information Agreement and the Settlement Agreement, each of which Dr. Montgomery signed and agreed to be bound thereto. True and correct copies of Nanogen's Employee Handbook and Dr. Montgomery's signed acknowledgement thereof are attached hereto as Exhibit "D" and incorporated herein by reference.

40. Dr. Montgomery knew, had reason to know, and continues to know that he acquired Proprietary Information, including trade secrets, from Nanogen, that he had a confidential relationship with Nanogen and that he had and continues to have an obligation to maintain the secrecy of and not to use or disclose Nanogen's Proprietary Information without Nanogen's consent.

41. As a senior level scientist at Nanogen, Dr. Montgomery promised Nanogen that he would disclose to Nanogen his research and any technology developed as a result of research performed by him, alone or together with other Nanogen employees, and that he would assign to Nanogen all rights in any technology or inventions developed or based upon proprietary information he acquired during his employment at Nanogen. Based upon Dr. Montgomery's promises and the confidential relationship existing between Nanogen and Dr. Montgomery, Nanogen depended on Dr. Montgomery to keep his promises so that Nanogen could retain rightful ownership of any such technology or inventions.

42. On information and belief, Dr. Montgomery breached the confidential relationship by using and disclosing Nanogen's Proprietary Information in the '302 Patent, the '221 PCT and '688 PCT for his own benefit and the benefit of CombiMatrix.

43. As a result of Dr. Montgomery's breach of the confidential relationship, Dr. Montgomery wrongfully gained ownership of the technology disclosed and claimed in the '302 Patent, the '221 PCT and the '688 PCT, and potentially in other foreign and domestic patents and/or applications, and commercially exploited the technology for his own personal gain and for the gain of CombiMatrix.

44. As a result of Dr. Montgomery's breach of the confidential relationship, Nanogen has been damaged by the loss of, including but not limited to, rights to the inventions disclosed and claimed in the '302 Patent, the '221 PCT and the '688 PCT, and potentially other domestic and foreign patents and applications, and all revenue derived therefrom, and the '302 patent, the '221 PCT and the '688 PCT themselves, and all revenue derived therefrom.

45. Dr. Montgomery was required to maintain Nanogen's Proprietary Information as secret and had agreed to refrain from misappropriating any of Nanogen's trade secrets by each of his agreement with Nanogen company policy, his Proprietary Information Agreement and the Settlement Agreement.

46. Dr. Montgomery knew, had reason to know, and continues to know that he acquired Proprietary Information, including trade secrets, from Nanogen either through or related to his employment at Nanogen, and that he had and continues to have a duty to maintain the secrecy and limit the use and disclosure of those trade secrets.

47. Nanogen actively sought to protect its trade secrets, and other Proprietary Information including the technology disclosed and claimed in the '302 Patent, the '221 PCT and '688 PCT, from unauthorized use and disclosure, including by Dr. Montgomery after the termination of Dr. Montgomery's employment with Nanogen. Nanogen was not aware of the misappropriation of Nanogen's trade secrets and other Proprietary Information by Dr. Montgomery and CombiMatrix until after the issuance of the '302 Patent, and publication of the '221 PCT and '688 PCT.

48. The technology disclosed and claimed in the '302 Patent, the '221 PCT and the '688 PCT was valuable as trade secrets, as possession of the trade secret information allowed Dr. Montgomery, personally and on behalf of CombiMatrix, to further develop the technology

described therein, obtain financing for development of a business designed to exploit the technology, seek patent protection, potentially license the invention to other business entities for use, and potentially market products derived from the technology to customers. The '302 Patent, the '221 PCT and the '688 PCT are disclosed as valuable intellectual property in CombiMatrix's November 22, 2000 S-1 filing.

49. Nanogen has made substantial expenditures in investigating and developing the technology disclosed and claimed in the '302 Patent, the '221 PCT and the '688 PCT.

50. Dr. Montgomery breached Nanogen's trust and confidence, Nanogen's company policies, the Proprietary Information Agreement and the Settlement Agreement. Further, Dr. Montgomery violated Nanogen's proprietary rights in its trade secrets and Proprietary Information by using them for Dr. Montgomery's own purposes and/or those of CombiMatrix, without Nanogen's consent.

51. Dr. Montgomery misappropriated Nanogen's trade secrets by disclosing and using Nanogen's trade secrets and Proprietary Information to CombiMatrix without Nanogen's permission or consent, including without limitation in the preparation and filing of the application for the '302 Patent, and the publication of the '302 Patent, the '221 PCT and the '688 PCT.

52. On information and belief, Dr. Montgomery, for himself personally and on behalf of CombiMatrix, misappropriated Nanogen's trade secrets and Proprietary Information at little or no cost to himself or CombiMatrix, thereby significantly reducing the cost of determining the feasibility and the cost of initial development of the subject matter of the '302 Patent, the '221 PCT and the '688 PCT.

53. Nanogen is informed and believes, and thereon alleges, that Dr. Montgomery for himself and on behalf of CombiMatrix, had the express intent of misappropriating Nanogen's Proprietary Information, including its trade secrets, and intended to use and currently is using Nanogen's Proprietary Information for his own and CombiMatrix's commercial advantage to compete directly with Nanogen and/or to destroy Nanogen's competitive advantage in the marketplace, without incurring the expense and time necessary to research and develop their own technology separate and apart from that taken from Nanogen.

54. Nanogen is informed and believes, and thereon alleges, that Dr. Montgomery, for himself personally and on behalf of CombiMatrix, knowingly, willfully and deliberately misappropriated Nanogen's trade secrets and other Proprietary Information with full knowledge of the secret and confidential nature and commercial value of such information. Nanogen further is informed and believes, and thereon alleges, that Dr. Montgomery's conduct was and is willful, malicious, despicable and in conscious disregard of Nanogen's rights and, therefore, Nanogen is entitled to an award of punitive damages in an amount not exceeding twice any award made for actual loss and for unjust enrichment, and to reasonable attorneys' fees, all pursuant to the California Uniform Trade Secrets Act, California Civil Code §§ 3426 *et seq.*

55. Nanogen is informed and believes, and thereon alleges, that Dr. Montgomery's wrongful use and exploitation of Nanogen's trade secrets and other Proprietary Information has already damaged Nanogen and threatens to and will continue to damage Nanogen and cause it irreparable harm unless restrained by this Court, and Nanogen is without an adequate remedy that could fully compensate it at law.

56. Nanogen is entitled to all available damages under California Civil Code § 3426.3(a) and (b).

57. The use and disclosures by Dr. Montgomery, for himself personally and on behalf of CombiMatrix, as described above were willful and malicious, justifying the award of exemplary damages under California Civil Code § 3426.3(c).

58. Defendant Dr. Montgomery had a duty to assign rights in this technology to Nanogen as required by Nanogen company policy and his Proprietary Information Agreement. Instead, Dr. Montgomery assigned rights in the technology to CombiMatrix.

59. As the correct assignee of the Nanogen Inventors and Dr. Montgomery while employed at Nanogen, Nanogen is entitled to an undivided interest in all rights, title and interest as co-owner and tenant-in-common in and to the '302 Patent, the '221 PCT and the '688 PCT and in and to any domestic or foreign applications or issued domestic or foreign patents claiming priority therefrom or relating thereto.

60. Nanogen is entitled to have this Court order Dr. Montgomery to assign any and all rights he may have in the '302 Patent, '221 PCT and the '688 PCT to Nanogen.

61. Nanogen is entitled to have this Court enjoin Dr. Montgomery from further disclosure and use of Nanogen's trade secret and other Proprietary Information, including from further domestic and foreign patent filings naming Dr. Montgomery as the inventor or applicant.

FOURTH CAUSE OF ACTION

(Misappropriation of Trade Secrets and Injunction Under the California Uniform Trade Secrets Act, Cal. Civil Code §§ 3426 et seq. (by Defendant CombiMatrix))

62. Nanogen hereby refers to and incorporates by reference Paragraphs 1 through 61, inclusive, of this Complaint.

63. By virtue of Dr. Montgomery's use of Nanogen's Proprietary Information, including Nanogen trade secret information, such information was acquired from Nanogen by improper means.

64. On information and belief, CombiMatrix acquired such Nanogen trade secret and other Proprietary Information when it knew or had reason to know that such information was acquired from Nanogen by improper means. Dr. Montgomery was an officer of CombiMatrix and knew that the Nanogen trade secret and Proprietary Information was acquired by improper means. Dr. Montgomery's knowledge is imputed to CombiMatrix.

65. CombiMatrix is deriving substantial economic benefit from such Nanogen trade secret and other Proprietary Information.

66. The technology disclosed and claimed in the '302 Patent, the '221 PCT and the '688 PCT was valuable as trade secrets, as possession of the trade secret information allowed CombiMatrix to further develop the technology described therein, obtain financing for development of a business designed to exploit the technology, seek patent protection, potentially license the invention to other business entities for use, and potentially market products derived from the technology to customers. The '302 Patent, the '221 PCT and the: '688 PCT are disclosed as valuable intellectual property in CombiMatrix's November 22, 2000 S-1 filing.

67. CombiMatrix violated Nanogen's proprietary rights in its trade secrets and Proprietary Information by using them for their own purposes without Nanogen's consent.

68. CombiMatrix misappropriated Nanogen's trade secrets by acquiring Nanogen's trade secrets and proprietary information from or through Dr. Montgomery without Nanogen's permission or consent, including without limitation in the preparation and filing of the application for the '302 Patent, and the publication of the '302 Patent, the '221 PCT and the '688 PCT.

69. On information and belief, CombiMatrix misappropriated Nanogen's trade secrets and Proprietary Information at little or no cost to itself, thereby significantly reducing the cost of determining the feasibility and the cost of initial development of the subject matter of the '302 Patent, the '221 PCT and the '688 PCT.

70. Nanogen is informed and believes, and thereon alleges, that CombiMatrix had the express intent of misappropriating Nanogen's Proprietary Information, including its trade secrets, and intended to acquire such for its own commercial advantage to compete directly with Nanogen and/or to destroy Nanogen's competitive advantage in the marketplace, without incurring the expense and time necessary to research and develop their own technology separate and apart from that taken from Nanogen.

71. Nanogen is informed and believes, and thereon alleges, that CombiMatrix knowingly, willfully and deliberately misappropriated Nanogen's trade secrets and other Proprietary Information with full knowledge of the secret and confidential nature and commercial value of such information. Nanogen further is informed and believes, and thereon alleges, that CombiMatrix's conduct was and is willful, malicious, despicable and in conscious disregard of Nanogen's rights and, therefore, Nanogen is entitled to an award of punitive damages in an amount not exceeding twice any award made for actual loss and for unjust enrichment, and to reasonable attorneys' fees, all pursuant to the California Uniform Trade Secrets Act, California Civil Code §§ 3426 *et seq.*

72. Nanogen is informed and believes, and thereon alleges, that CombiMatrix's wrongful acquisition and exploitation of Nanogen's trade secrets and other Proprietary Information has already damaged Nanogen and threatens to and will continue to damage Nanogen and cause it

irreparable harm unless restrained by this Court, and Nanogen is without an adequate remedy that could fully compensate it at law.

73. Nanogen is entitled to all available damages under California Civil Code § 3426.3(a) and (b).

74. The trade secret acquisition and exploitation by CombiMatrix as described above were willful and malicious, justifying the award of exemplary damages under California Civil Code § 3426.3(c).

75. Defendant Dr. Montgomery had a duty to assign rights in this technology to Nanogen as required by Nanogen company policy and his Proprietary Information Agreement. Instead, Dr. Montgomery assigned rights in the technology to CombiMatrix.

76. Nanogen is entitled to have the Court order transfer of assignment of any and all rights CombiMatrix may have in the '302 Patent, the '221 PCT and the '688 PCT, to Nanogen.

77. Nanogen is entitled to have the Court enjoin CombiMatrix from further exploitation of Nanogen's trade secret and other Proprietary Information, including from further domestic and foreign patent filings naming CombiMatrix as the applicant or assignee.

FIFTH CAUSE OF ACTION

(Breach of Written Contract Re: Proprietary Information Agreement (Against Defendant Dr. Montgomery))

78. Nanogen hereby refers to and incorporates by reference paragraphs 1 through 77, inclusive, of this Complaint.

79. Under the Proprietary Information Agreement with Nanogen, and by his signed acknowledgement of the company policies set forth in Nanogen's Employee Handbook, Dr. Montgomery assumed contractual obligations to Nanogen in connection with his employment. Dr. Montgomery's obligations to Nanogen include, without limitation: 1) his agreements to assign technology and inventions to Nanogen; 2) his agreement to maintain in confidence and not disclose Nanogen Proprietary Information; 3) his agreement to disclose inventions to Nanogen; 4) his agreement to return Nanogen documents; 5) the duty to record his scientific research, results and conclusions in laboratory notebooks issued by Nanogen; and 6) his duty to make regular reports

and presentations to his supervisors and colleagues at Nanogen summarizing his scientific research, results and conclusions. Dr. Montgomery's Proprietary Information Agreement is attached hereto as Exhibit A and incorporated herein by reference.

80. Dr. Montgomery breached his written Proprietary Information Agreement and Nanogen's company policies by failing to perform each of his obligations as set forth in the above paragraph, including failing disclose some amount of his research and his contributions to the technology disclosed and claimed in the '302 Patent, the '221 PCT and the '688 PCT in his laboratory notebooks, reports or presentations, and by using Nanogen's trade secrets and other Proprietary Information for his own purposes and/or those of CombiMatrix during and after his employment, including by the use and disclosure related to the '302 Patent, the '221 PCT and the '688 PCT, and by failing to assign to Nanogen ownership rights in this technology and in any patents or patent applications in which this technology or any other Nanogen trade secret or Proprietary Information is disclosed.

81. Nanogen has performed all conditions, covenants and promises required on its part in accordance with the terms and conditions of the Proprietary Information Agreement and the Employee Handbook.

82. Nanogen is informed and believes, and thereon alleges that Dr. Montgomery's acts and omissions as alleged in this Complaint constitute material breaches of his obligations under the Proprietary Information Agreement and the Employee Handbook Acknowledgement, each of which were voluntarily agreed to and signed by Dr. Montgomery.

83. As a direct and proximate result of Dr. Montgomery's secretive and material breaches of Nanogen's company policies and the Proprietary Information Agreement and the facts here alleged, Nanogen has suffered and will continue to suffer substantial damages by the loss of, including but not limited to, rights to the technology disclosed and claimed in the '302 Patent, the '221 PCT and the '688 PCT, and all revenue derived therefrom, and the '302 Patent, the '221 PCT and the '688 PCT themselves, and all revenue derived therefrom. Nanogen has suffered damages in an amount to be proven at trial, but believed to be substantial.

84. Nanogen was not aware nor should it reasonably have been aware of its potential causes of action against Dr. Montgomery, including its breach of written contract claim regarding the Proprietary Information Agreement, due to the secretive nature of Dr. Montgomery's breach.

SIXTH CAUSE OF ACTION

(Breach of Written Contract Re: Settlement Agreement (Against Defendant Dr. Montgomery))

85. Nanogen hereby refers to and incorporates by reference paragraphs 1 through 84, inclusive, of this Complaint.

86. Under the Settlement Agreement Dr. Montgomery voluntarily signed to terminate his lawsuit against Nanogen, Dr. Montgomery again promised, and was aware of his obligation, to protect in confidence, and not to disclose or use in competition with Nanogen and/or in any way to Nanogen's detriment, any research conducted or technology developed at Nanogen by Dr. Montgomery himself and/or by other Nanogen employees and/or any Nanogen trade secrets or other Proprietary Information of which Dr. Montgomery became aware during his employment.

87. On information and belief, Dr. Montgomery materially breached the written Settlement Agreement with Nanogen by, among other actions by breaching: 1) his covenant not to compete, as defined in the Settlement Agreement; and 2) his agreement not to misappropriate trade secrets; including by failing to protect in confidence, disclosing and/or using in competition with Nanogen, the subject matter in the '302 Patent, the '221 PCT and the '688 PCT to Nanogen's detriment, as well as research conducted or technology developed at Nanogen by Dr. Montgomery himself and/or by other Nanogen employees, and/or any Nanogen trade secrets or other Proprietary Information of which Dr. Montgomery became aware during his employment.

88. Nanogen has performed all conditions, covenants and promises required on its part in accordance with the terms and conditions of the Settlement Agreement.

89. Nanogen is informed and believes, and thereon alleges that Dr. Montgomery's acts and omissions as alleged in this Complaint constitute material breaches of his obligations under the Settlement Agreement, which was voluntarily agreed to and signed by Dr. Montgomery.

90. As a direct and proximate result of Dr. Montgomery's secretive and material breaches of Nanogen's company policies, the Settlement Agreement and the facts here alleged, Nanogen has suffered and will continue to suffer substantial damages by the loss of, including but not limited to, rights to the technology disclosed and claimed in the '302 Patent, the '221 PCT and the '688 PCT, and all revenue derived therefrom, and the '302 Patent, the '221 PCT and the '688 PCT themselves, and all revenue derived therefrom. Nanogen has suffered damages in an amount to be proven at trial, but believed to be substantial.

91. Nanogen was not aware nor should it reasonably have been aware of its potential causes of action against Dr. Montgomery, including its breach of written contract claim regarding the Settlement Agreement, due to the secretive nature of Dr. Montgomery's breach.

SEVENTH CAUSE OF ACTION

(Inducing Breach Of Written Contract (Against Defendant CombiMatrix))

92. Nanogen hereby refers to and incorporates by reference paragraphs 1 through 91, inclusive, of this Complaint.

93. On information and belief, Defendant CombiMatrix knew of Dr. Montgomery's former employment at Nanogen and of the existence of the Proprietary Information Agreement and Settlement Agreement between Dr. Montgomery and Nanogen.

94. On information and belief, CombiMatrix, with such knowledge of the Agreements and of Nanogen's reliance on these Agreements, intentionally acted to induce Dr. Montgomery to disclose and use Nanogen's trade secrets and other Proprietary Information and to disclose and use the '302 Patent, the '221 PCT and the '688 PCT and the technology described therein, and to assign the '302 Patent, the '221 PCT and '688 PCT to CombiMatrix and thereby breach Dr. Montgomery's Proprietary Information Agreement and Settlement Agreement with Nanogen.

95. As a direct and proximate result of CombiMatrix's inducement to cause Dr. Montgomery's secretive and material breaches of the Proprietary Information Agreement and Settlement Agreement and the facts here alleged, Nanogen has suffered and will continue to suffer substantial damages by the loss of, including but not limited to, rights to the technology disclosed

and claimed in the '302 Patent, the '221 PCT and the '688 PCT and all revenue derived therefrom, and the '302 Patent, the '221 PCT and the '688 PCT themselves and all revenue derived therefrom. Nanogen has suffered damages in an amount to be proven at trial, but believed to be substantial.

96. Nanogen was not aware nor should it reasonably have been aware of its potential causes of action against CombiMatrix, including its inducing breach of written contract claims, due to the secretive nature of CombiMatrix's breach.

EIGHTH CAUSE OF ACTION

(Breach Of Implied Covenant Of Good Faith And Fair Dealing (Against Defendant Dr. Montgomery))

97. Nanogen hereby refers to and incorporates by reference paragraphs 1 through 96, inclusive, of this Complaint.

98. By virtue of Dr. Montgomery's employment agreement with Nanogen, his Acknowledgement of the Employee Handbook, the Proprietary Information Agreement and the Settlement Agreement with Nanogen, Dr. Montgomery impliedly covenanted to act toward and deal with Nanogen fairly and in good faith.

99. Dr. Montgomery's acts and omissions as alleged above constituted a breach of the covenant of good faith and fair dealing implied in Dr. Montgomery's employment, Proprietary Information Agreement and Settlement Agreement with Nanogen.

100. As a direct and proximate result of Dr. Montgomery's breach of the implied covenant of good faith and fair dealing, Nanogen has suffered and will continue to suffer substantial damages by the loss of, including but not limited to, rights to the inventions disclosed and claimed in the '302 Patent, the '221 PCT and the '688 PCT, and all revenue derived therefrom, and the '302 Patent, the '221 PCT and the '688 PCT themselves, and all revenue derived therefrom. Nanogen has suffered damages in an amount to be proven at trial, but believed to be substantial.

101. Nanogen was not aware nor should it reasonably have been aware of its potential causes of action against Dr. Montgomery, including its breach of implied covenant of good faith and fair dealing claim, due to the secretive nature of Dr. Montgomery's breach.

NINTH CAUSE OF ACTION

(Intentional Interference With Prospective Economic Advantage (Against Both Defendants))

102. Nanogen hereby refers to and incorporates by reference paragraphs 1 through 101, inclusive, of this Complaint.

103. Upon information and belief, despite his obligations to do so, Dr. Montgomery has failed to disclose to Nanogen that he and/or the Nanogen Inventors made or derived inventions during his employment at Nanogen and failed to assign to Nanogen patents and applications for patents claiming such inventions filed after termination of his employment at Nanogen. Instead, Dr. Montgomery disclosed, CombiMatrix acquired, and both Defendants used and commercially exploited Nanogen's trade secrets and/or other Proprietary Information, including the technology disclosed and claimed in the '302 Patent, the '221 PCT and the '688 PCT, as if they were the rightful owners. These actions resulted in the denial of Nanogen's rights in the technology disclosed and claimed in the '302 Patent, the '221 PCT and the '688 PCT, and potentially other patent applications, and the loss of Nanogen's valuable trade secrets.

104. As a result of Dr. Montgomery's secretive and material breach of his obligations to Nanogen and the facts alleged herein, Nanogen has suffered and will continue to suffer substantial economic damages by the loss of, including but not limited to, rights to the inventions disclosed in the '302 Patent, the '221 PCT and the '688 PCT, and all revenue derived therefrom, and the '302 Patent, the '221 PCT and the '688 PCT themselves, and all revenue derived therefrom. Nanogen has suffered damages in an amount to be proven at trial, but believed to be substantial.

105. Defendant CombiMatrix intentionally and wrongfully acted to disrupt the established relationship between Nanogen and Dr. Montgomery by causing Dr. Montgomery to disclose and assign an alleged ownership interest in the technology disclosed and claimed in the '302 Patent, the '221 PCT and the '688 PCT to CombiMatrix, contrary to Nanogen's company policies, the Proprietary Information Agreement and the Settlement Agreement, which require Dr. Montgomery to maintain the confidentiality and assign ownership of this technology to Nanogen.

106. Dr. Montgomery's commitments and promises to Nanogen were valuable to Nanogen, as they entitled Nanogen to ownership of '302 Patent, the '221 PCT and the '688 PCT, and potentially other patents or applications, as well as rights to the technology disclosed therein and to commercialize and sub-license the technology disclosed therein.

107. As a direct and proximate result of the actions of Dr. Montgomery and CombiMatrix, Nanogen has suffered and will continue to suffer substantial damages by the loss of, including but not limited to, rights to the technology and inventions disclosed and claimed in '302 Patent, the '221 PCT and the '688 PCT and all revenue derived therefrom, and '302 Patent, the '221 PCT and the '688 PCT themselves and all revenue derived therefrom. Nanogen has suffered damages in an amount to be proven at trial, but believed to be substantial.

108. On information and belief, Dr. Montgomery and CombiMatrix misappropriated Nanogen's trade secrets and Proprietary Information at little or no cost to themselves, thereby significantly reducing the cost of determining the feasibility and the cost of initial development of the subject matter of the '302 Patent, the '221 PCT and the '688 PCT.

109. The technology disclosed and claimed in the '302 Patent, the '221 PCT and the '688 PCT was valuable to Nanogen as trade secrets, as possession of that information allowed Dr. Montgomery and CombiMatrix to further develop the technology, obtain financing for development of a business designed to exploit the technology, seek patent protection, potentially license the invention to other business entities for use, and potentially market products derived from the technology to customers. Each of these actions gave the Defendants an actual or prospective economic advantage that should have inured to Nanogen exclusively.

110. Nanogen is informed and believes, and thereon alleges, that Dr. Montgomery and CombiMatrix had the express intent of misappropriating Nanogen's Proprietary Information, including its trade secrets, and acquired and intended to use and currently is using Nanogen's Proprietary Information for their own commercial advantage to compete directly with Nanogen and/or to destroy Nanogen's competitive advantage in the marketplace and prospective economic advantage, without incurring the expense and time necessary to research and develop their own technology separate and apart from that taken from Nanogen.

111. Nanogen was not aware nor should it reasonably have been aware of its potential causes of action against Dr. Montgomery and CombiMatrix, including the intentional interference with potential economic advantage cause of action, due to the secretive and fraudulent nature of Defendants' tortious conduct.

TENTH CAUSE OF ACTION

(Unfair Competition under the Common Law and California Business & Professions Code § 17200 (Against Both Defendants))

112. Nanogen hereby refers to and incorporates by reference paragraphs 1 through 111, inclusive, of this Complaint.

113. By unlawfully, unfairly and/or intentionally acting to interfere with Nanogen's contractual relations and prospective economic advantage by using, disclosing, commercially exploiting and claiming ownership of Nanogen's trade secrets and other Proprietary Information, and other deliberate acts and omissions as described above, Dr. Montgomery, for himself and on behalf of CombiMatrix, and CombiMatrix engaged in improper means to obtain a business benefit to the detriment of Nanogen.

114. Nanogen is informed and believes, and thereon alleges, that Dr. Montgomery's actions, for himself and on behalf of CombiMatrix, and CombiMatrix's actions constitute unfair competition against Nanogen under California common law which has resulted and threatens to continue to result in damages and other further and substantial irreparable harm to Nanogen. Nanogen further is informed and believes, and thereon alleges, that Dr. Montgomery, for himself and on behalf of CombiMatrix, and CombiMatrix acted unlawfully, unfairly and/or willfully, maliciously, despicably and in conscious disregard of Nanogen's rights entitling Nanogen to punitive damages under California Civil Code § 3294.

115. Nanogen is informed and believes, and thereon alleges, that Dr. Montgomery's actions, for himself and on behalf of CombiMatrix, and CombiMatrix's actions constitute a pattern of business unfair trade practices in violation of California Business and Professions Code § 17200 *et seq.* which has resulted in and continues to cause and threaten to cause Nanogen substantial and irreparable harm.

116. As a direct and proximate result of Dr. Montgomery's unfair competition, for himself and on behalf of CombiMatrix, and CombiMatrix's unfair competition, Nanogen has suffered and will continue to suffer substantial damages by the loss of, including but not limited to, rights to the inventions disclosed and claimed in the '302 Patent, the '221 PCT and the '688 PCT and all revenue derived therefrom, and the '302 Patent, the '221 PCT and the '688 PCT themselves and all revenue derived therefrom. Nanogen has suffered damages in an amount to be proven at trial, but believed to be substantial.

117. Nanogen is informed and believes that each of the acts and omissions by Defendants complained of in this cause of action constitutes an act done willfully and with malice, thereby supporting the award of exemplary damages.

ELEVENTH CAUSE OF ACTION

(Unjust Enrichment (Against Both Defendants))

118. Nanogen hereby refers to and incorporates by reference paragraphs 1 through 117, inclusive, of this Complaint.

119. Nanogen did not grant to Defendants, and Defendants did not bargain for or receive, any ownership rights to the technology disclosed and claimed in the '302 Patent, the '221 PCT, the '688 PCT or any and all other domestic or foreign issued patents or applications that encompass the Nanogen Inventors' contributions and/or Dr. Montgomery's contributions to this technology while employed at Nanogen.

120. On information and belief, Defendants have and/or will unjustly receive money from venture capitalists, sale of stock to the public, sales, collaborative agreements, and/or sub-license fees as a result of the misappropriation of trade secrets and/or other Proprietary Information, the unauthorized uses of the technology disclosed and claimed in the '302 Patent, the '221 PCT, the '688 PCT to which Nanogen claims ownership, and other wrongful acts set forth above.

121. Allowing Defendants to retain any funds received as a result of their wrongful acts would unjustly benefit Defendants to the damage, detriment and exclusion of Nanogen.

122. Defendants have wrongfully detained the property consisting of the technology disclosed and claimed in the '302 Patent, the '221 PCT, the '688 PCT, and all remedies derived therefrom, and potentially other patents and applications, within the meaning of California Civil Code § 2223 and thus are involuntary trustees thereof for the benefit of Nanogen.

123. Defendants have gained control over the technology disclosed '302 Patent, the '221 PCT, the '688 PCT, and the patent and applications themselves, by accident, mistake, undue influence, the violation of a trust, or other wrongful acts within the meaning of California Civil Code § 2224, do not possess some other and better right to the technology and the Patent and applications, and are involuntary trustees of the Patent and applications, and potentially other domestic and foreign patents and applications, and the technology disclosed therein, for the benefit of Nanogen who would otherwise have gained ownership of each of them.

124. Accordingly, Nanogen requests that this Court declare Dr. Montgomery and/or CombiMatrix involuntary trustees of the technology disclosed and claimed in the '302 Patent, the '221 PCT, the '688 PCT, and any and all domestic and foreign patents and applications related thereto, and the patent and applications themselves, in constructive trust for the benefit of Nanogen.

TWELFTH CAUSE OF ACTION

(Constructive Trust (Against Both Defendants))

125. Nanogen hereby refers to and incorporates by this reference paragraphs 1 through 124, inclusive, of this Complaint.

126. Nanogen is entitled to a constructive trust over the trust res which consists of (1) the technology and/or inventions disclosed and claimed in the '302 Patent, the '221 PCT and the '688 PCT and any and all domestic and foreign patents and applications related thereto, and all revenue derived therefrom and (2) the patents and/or applications themselves and all revenue derived therefrom.

127. On information and belief, Dr. Montgomery and CombiMatrix are constructive trustees over the trust res described in Paragraph 126 above because Defendants' acquisition of the trust res resulted from Dr. Montgomery's wrongful failure to disclose and assign the technology

and/or inventions constituting the trust res to Nanogen, and because Nanogen is entitled to possession and ownership of the trust res due to its development by Nanogen employees at Nanogen.

128. Nanogen was not aware nor should it reasonably have been aware of its potential causes of action against Dr. Montgomery and CombiMatrix, including its claim for constructive trust, due to the secretive and fraudulent nature of Defendants' tortious conduct.

129. Accordingly, Nanogen requests that the Court declare Dr. Montgomery and/or CombiMatrix involuntary trustees of the technology disclosed and claimed in the '302 Patent, the '221 PCT, the '688 PCT, and any and all domestic and foreign patents and applications related thereto, and the patent and applications themselves, in constructive trust for the benefit of Nanogen.

THIRTEENTH CAUSE OF ACTION

(Injunctive Relief (Against Both Defendants))

130. Nanogen hereby refers to and incorporates by reference paragraphs 1 through 129, inclusive, of this Complaint.

131. Nanogen is entitled to an injunction preventing Dr. Montgomery and CombiMatrix, their agents, representatives, employees and all those acting in concert with or under any of them, from using or disclosing to third parties Nanogen's Proprietary Information, including Nanogen's trade secrets, inventions, and technical know-how.

132. Nanogen is entitled to an injunction compelling Dr. Montgomery and CombiMatrix to disclose to Nanogen all technology or inventions Dr. Montgomery made/makes, conceived/conceives, reduced/reduces to practice or developed/develops (in whole or in part, either alone or jointly with others) during or as a result of his employment with Nanogen.

133. Nanogen is entitled to an injunction compelling Dr. Montgomery and CombiMatrix to assign to Nanogen all technology or inventions Dr. Montgomery made/makes, conceived/conceives, reduced/reduces to practice or developed/develops (in whole or in part, either alone or jointly with others) during or as a result of his employment with Nanogen, including but not limited to the technology disclosed and claimed in the '302 Patent, the '221 PCT, the '688

PCT, and any and all domestic and foreign patents and applications related thereto, and the patent and applications themselves.

134. Nanogen is entitled to a preliminary injunction preventing Dr. Montgomery and CombiMatrix from licensing or transferring any rights in the '302 Patent, the '221 PCT, the '688 PCT, and any and all domestic and foreign patents and applications related thereto.

135. Defendants' wrongful conduct and threatened continued wrongful conduct, unless and until enjoined and restrained by order of this Court, will cause grave and irreparable harm to Nanogen in that Nanogen's competitive advantage will be diminished or destroyed.

136. Nanogen has no adequate remedy at law for the injuries it will suffer if Dr. Montgomery and CombiMatrix are not enjoined from their present course of conduct or it will be extremely difficult to ascertain the amount of compensation that would afford Nanogen adequate relief.

FOURTEENTH CAUSE OF ACTION

(Conversion of the '302 Patent, the '221 PCT and the '688 PCT (Against Both Defendants))

137. Nanogen hereby refers to and incorporates by this reference paragraphs 1 through 136, inclusive, of this Complaint.

138. In filing the patent application of the '302 Patent and allowing its issuance covering subject matter that was conceived and developed by the Nanogen Inventors alone or jointly with Dr. Montgomery, Dr. Montgomery omitted naming the Nanogen Inventors as inventors or co-inventors of the subject matter claimed in the '302 Patent. Through the omission of the Nanogen Inventors as inventors or co-inventors and upon the issuance of the '302 Patent and the publication of the '221 PCT and the '688 PCT, Dr. Montgomery actually interfered with and effectively denied Nanogen's ownership position, as the Nanogen Inventors' assignee, in the '302 Patent, the '221 PCT and the '688 PCT.

139. Defendants have from at least July, 1996 to the present day, exercised complete and absolute control over the '302 Patent, the '221 PCT, the '688 PCT, and the respected applications related thereto, and possibly other related patents and applications to the exclusion of Nanogen.

140. Nanogen, as assignee of the Nanogen Inventors and of Dr. Montgomery during his employment at Nanogen, has a right to possession of the '302 patent, as well as any patents resulting from the '221 PCT, the '688 PCT, and other related domestic and foreign patents and applications.

141. The denial of the legal recognition of the Nanogen Inventors as inventors or co-inventors has precluded Nanogen from further developing, marketing or licensing the technology disclosed and claimed in the '302 Patent, the '221 PCT and the '688 PCT in order to achieve commercialization and benefit therefrom. As a result, Nanogen has been and continues to be severely damaged by Defendants' conversion of the technology, patents and applications.

142. Defendant Dr. Montgomery has materially benefited by assigning his rights in the technology disclosed and claimed in the '302 Patent, the '221 PCT and the '688 PCT, the patents and applications themselves and potentially other patents and applications to CombiMatrix. Nanogen is informed and believes, and on that basis alleges, that CombiMatrix recompensed Dr. Montgomery as if Dr. Montgomery were the sole inventor and as such could grant exclusive rights to practice the invention.

143. Nanogen is entitled to compensatory damages for the injuries suffered as a result of the aforementioned conversion and resultant complete deprivation of Nanogen's property rights in the '302 Patent, the '221 PCT and the '688 PCT, the patents and applications themselves and all revenue derived therefrom.

144. Nanogen was not aware nor should it reasonably have been aware of its potential causes of action against Dr. Montgomery and CombiMatrix, including its conversion claim, due to the secretive and fraudulent nature of Defendants' tortious conduct.

PRAYER FOR RELIEF

WHEREFORE, Nanogen prays for relief as follows:

A. For correction of the inventorship to add the Nanogen Inventors to the '302 patent, the '221 PCT, and the '688 PCT, and related applications;

B. For Assignment of any and all rights of CombiMatrix Corporation and/or Dr. Montgomery in the '302 Patent, the '221 PCT, the '688 PCT and any related

patents or applications to Nanogen;

C. For an injunction permanently enjoining Defendants from further disclosure and use of any of Nanogen's trade secret information and Proprietary Information including that misappropriated and incorporated into the '302 Patent and related applications;

D. For an injunction preventing Dr. Montgomery, CombiMatrix, their agents, representatives, employees and all those acting in concert with or under any of them, from using or disclosing to third parties Nanogen's proprietary information, including Nanogen's trade secrets, inventions, and technical know-how;

E. For an injunction compelling Dr. Montgomery and CombiMatrix to disclose to Nanogen all technology or inventions Dr. Montgomery made, conceived, reduced to practice or developed (in whole or in part, either alone or jointly with others) during or as a result of his employment with Nanogen;

F. For an injunction compelling Dr. Montgomery and CombiMatrix to assign to Nanogen all technology or inventions Dr. Montgomery made, conceived, reduced to practice or developed (in whole or in part, either alone or jointly with others) during or as a result of his employment with Nanogen, including but not limited to the technology disclosed and claimed in the '302 Patent, the '221 PCT, the '688 PCT, and the patent and applications themselves;

G. For a preliminary injunction and permanent injunction preventing the Defendants from transferring or licensing any rights to the '302 patent, the '688 PCT or the '221 PCT or any related patents or applications, and from filing or causing to have filed any additional related patent applications;

H. For an award of damages to Nanogen adequate to compensate Nanogen for damages incurred as a result of Defendants' trade secret misappropriation, breach of written contracts, inducing breach of contracts, breach of implied covenant of good faith and fair dealing, intentional interference with prospective economic advantage, unjust enrichment and conversion;

I. For damages in an amount sufficient to compensate Nanogen for the damage caused

by the Defendants unfair competition under common law and California Business and Professions Code section 17200, including punitive damages under California Civil Code section 3294 and exemplary damages provided by 517206 of the California Business and Professions Code;

J. For exemplary damages under California Civil Code section 3426.3(c);

K. For attorneys fees under California Civil Code section 3426.4 and any other applicable law or contract; and

L. Any other damages or relief the Court may deem appropriate.

V. DEMAND FOR JURY TRIAL

Pursuant to Rule 38 of the Federal Rules of Civil Procedure, Plaintiff Nanogen respectfully demands a trial by jury on all issues that cannot be resolved as a matter of law.

DATED: November 28, 2000

BROBECK, PHLEGER & HARRISON LLP

By: _____

Douglas E. Olson
Jeffrey W. Guise
Peter R. Munson
Attorneys for Plaintiffs
NANOGEN, INC.

INDEX OF EXHIBITS

NANOGEN, INC

00cv2369JM(RBB)

-v-

DONALD D. MONTGOMERY, et al.

SEALED DOCUMENT

EXHIBIT A

US006093302A

United States Patent [19]

Montgomery

[11] Patent Number: 6,093,302

[45] Date of Patent: Jul. 25, 2000

[54] ELECTROCHEMICAL SOLID PHASE SYNTHESIS

[75] Inventor: **Donald D. Montgomery**, Millbrae, Calif.

[73] Assignee: **Combimatrix Corporation**, Burlingame, Calif.

[21] Appl. No.: **09/003,075**

[22] Filed: **Jan. 5, 1998**

[51] **Int. Cl.**[7] **C25D 5/02**

[52] **U.S. Cl.** **205/122**

[58] **Field of Search** 205/157, 162, 205/164, 122; 204/483, 485, 484

[56] **References Cited**

U.S. PATENT DOCUMENTS

4,764,263	8/1988	Gregory et al.	204/74
5,143,854	9/1992	Pirrung et al.	436/518
5,679,590	10/1997	Mori, et al. .	
5,810,989	9/1998	Kribak et al.	205/91

FOREIGN PATENT DOCUMENTS

2 703 359	10/1994	France .
WO 93/22480	11/1993	WIPO .
WO 95/12808	11/1995	WIPO .
WO 96/07917	3/1996	WIPO .
WO 97/11905	4/1997	WIPO .
WO 98/01221	1/1998	WIPO .

OTHER PUBLICATIONS

Gildea, Brian D. et al., "A Versatile Acid-labile Linker for modification of Synthetic BioMolecules" *Tetrahedron Letters* vol. 31:No.49:pp. 7095–7098 (1990) no month avail.

Livache, Thierry et al., "Polypyrrole DNA Chip on a Silicon Device: Example of Hepatitis C Virus Genotyping" *Analytical Biochemistry* 255: 188–194 (1998) no month avail.

Meo, Tommaso, et al., "Monoclonal antibody to the message sequence TryGly–Gly–Phe of optoid peptides exhibits the specificity requirements of mammalian optoid receptors", *Proc. Natl. Acad. Sci USA* 80: 4084–4088 (1983) Jul. 1983.

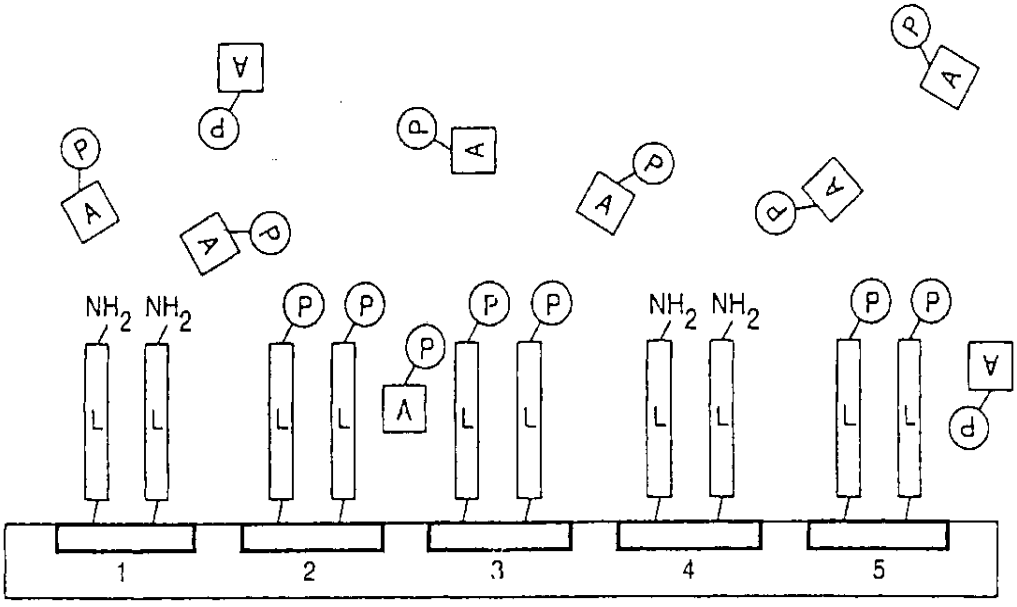
Merrifield, *J. Am. Chem. Soc.* 85:2149–2154 (1963) Jul. 20, 1963.

Primary Examiner—Kathryn Gorgos
Assistant Examiner—Erica Smith-Hicks
Attorney, Agent, or Firm—Albert P. Halluin; J. David Smith; Howrey & Simon

[57] ABSTRACT

A solid phase synthesis method for the preparation of diverse sequences of separate polymers or nucleic acid sequences using electrochemical placement of monomers or nucleic acids at a specific location on a substrate containing at least one electrode that is preferably in contact with a buffering or scavenging solution to prevent chemical crosstalk between electrodes due to diffusion of electrochemically generated reagents.

48 Claims, 39 Drawing Sheets



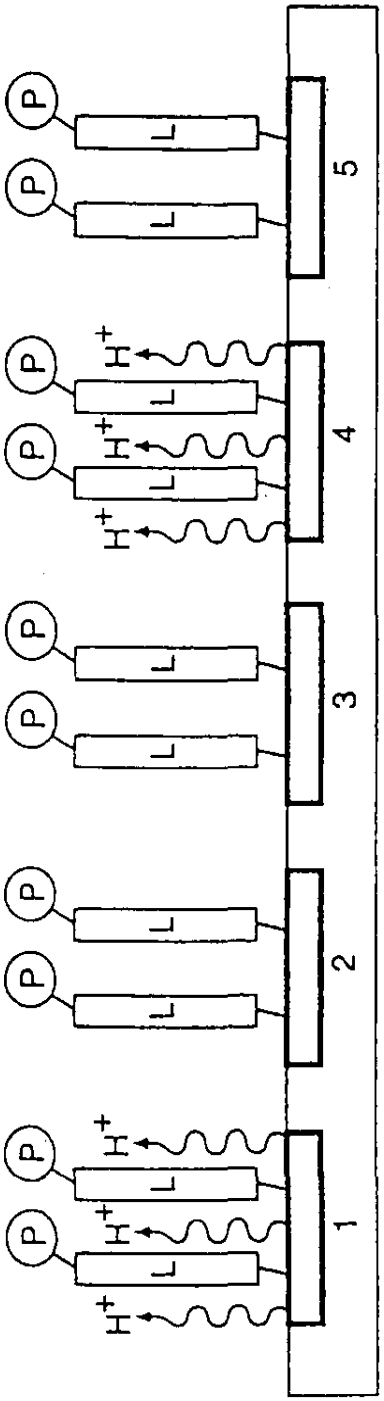


FIG. 1a

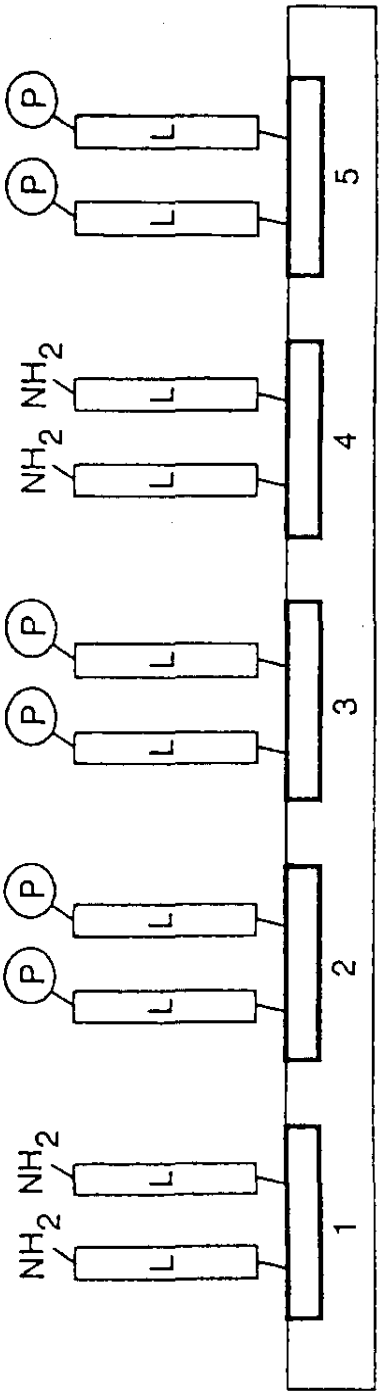


FIG. 1b

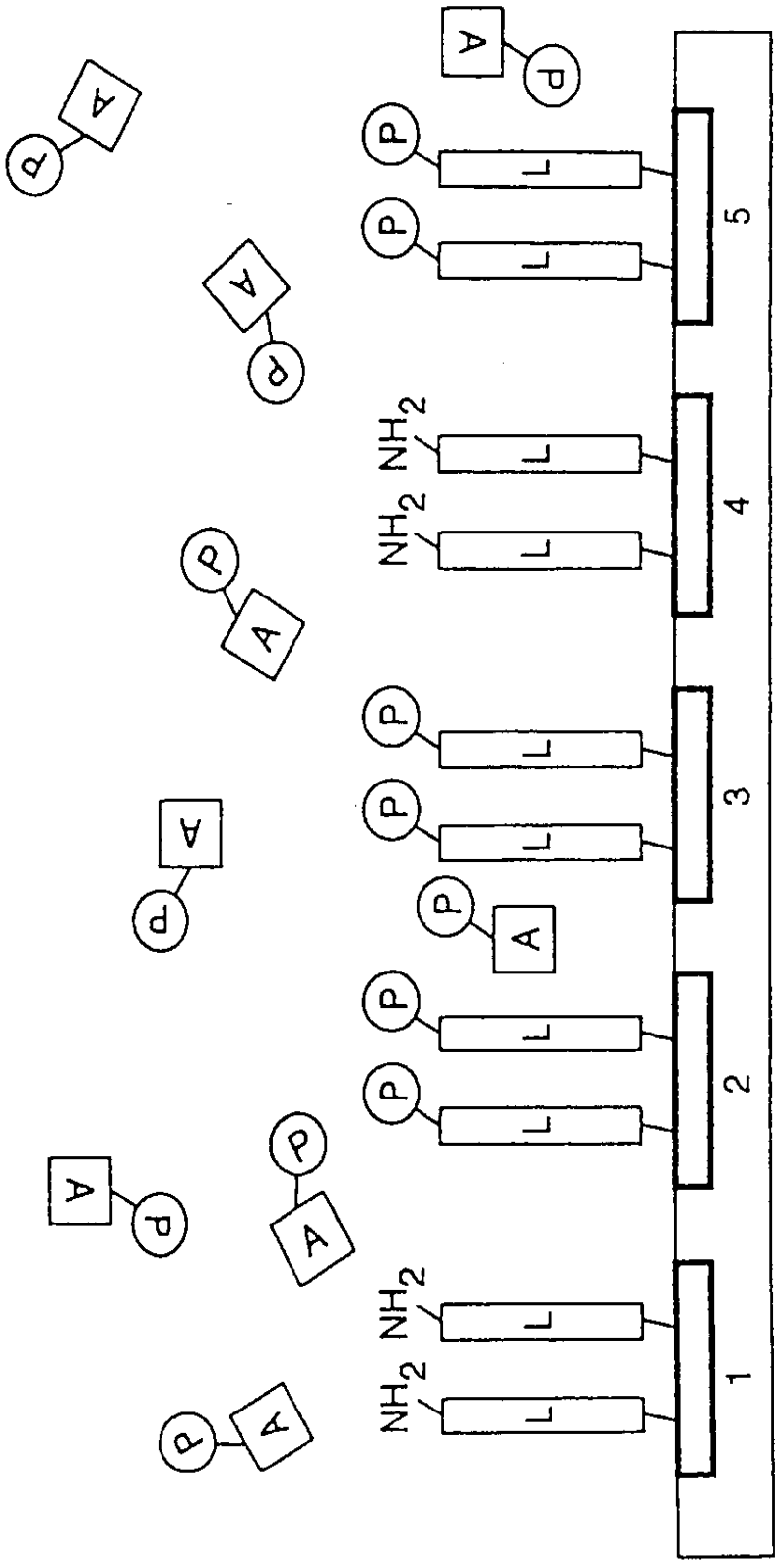


FIG. 2a

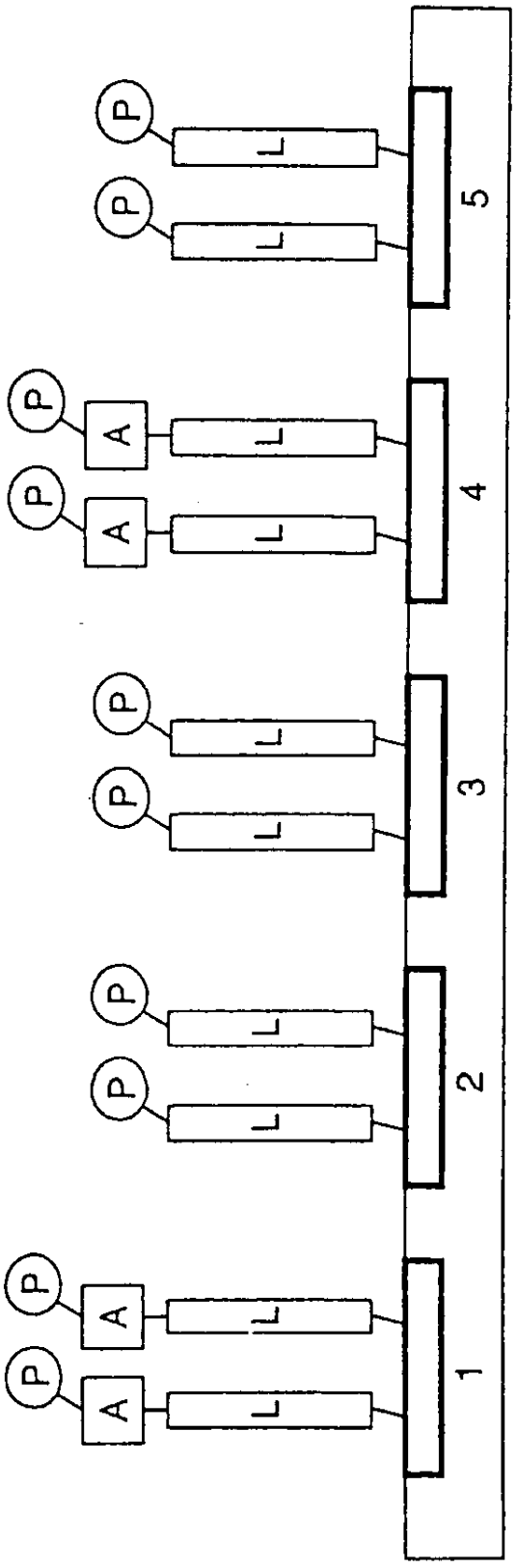


FIG. 2b

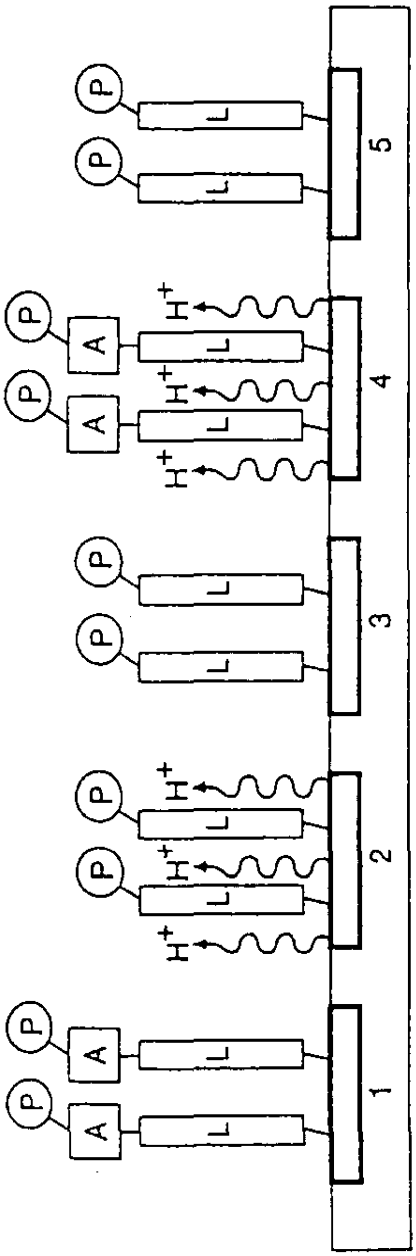


FIG. 3a

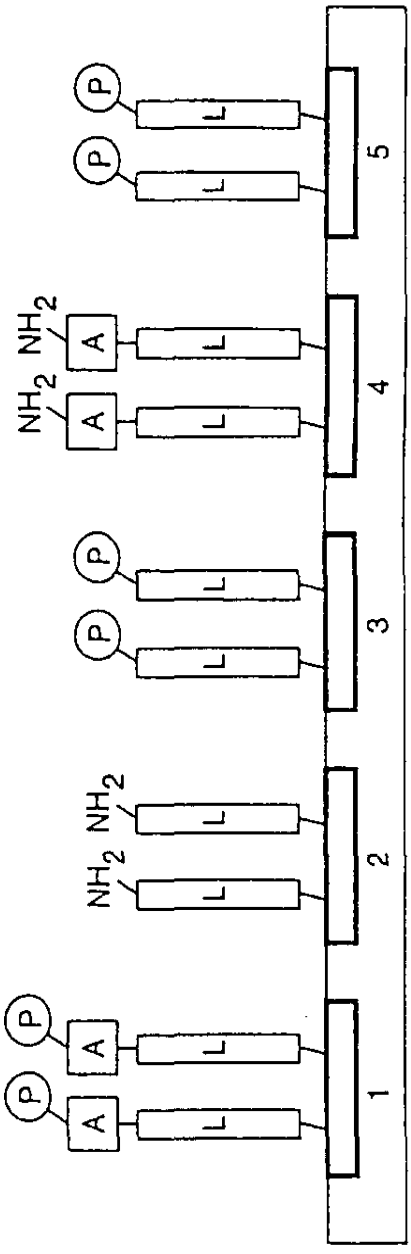


FIG. 3b

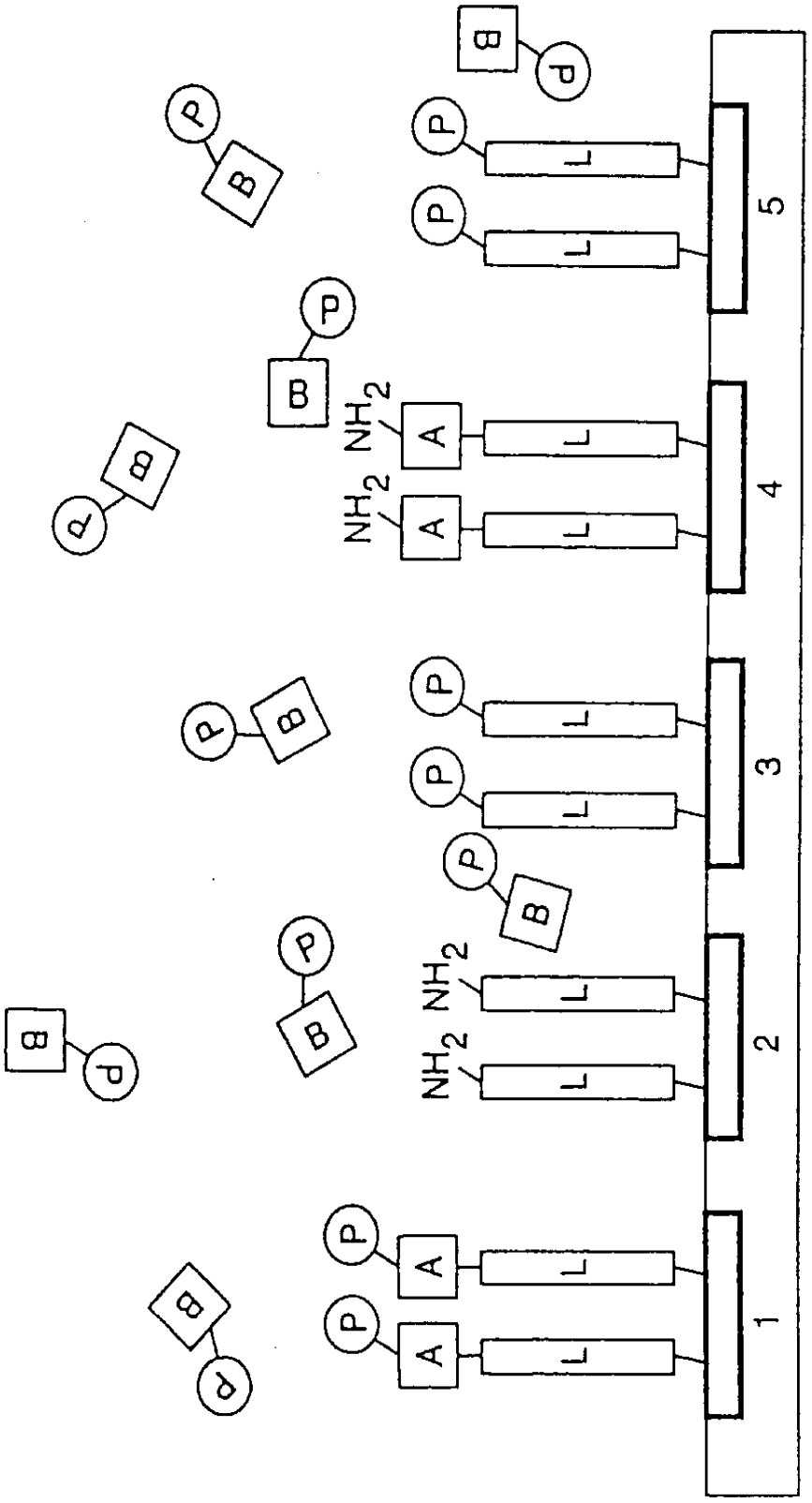


FIG. 4a

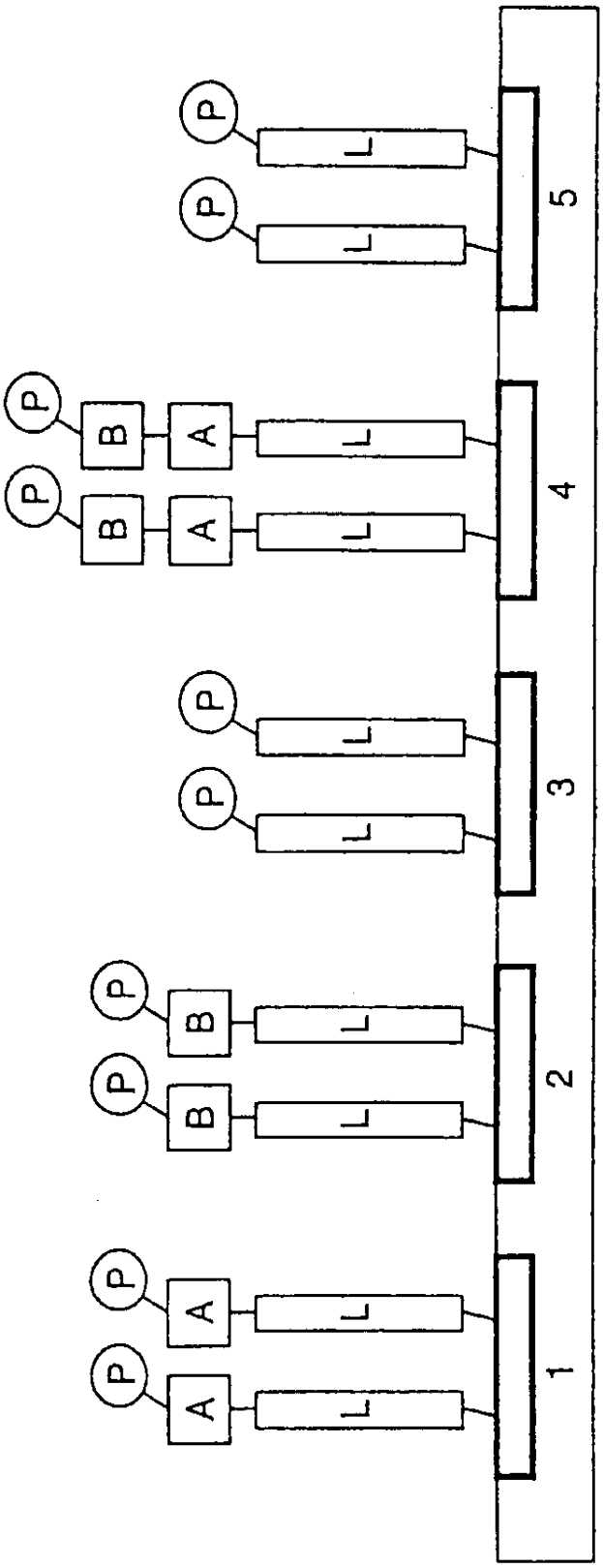


FIG. 4b

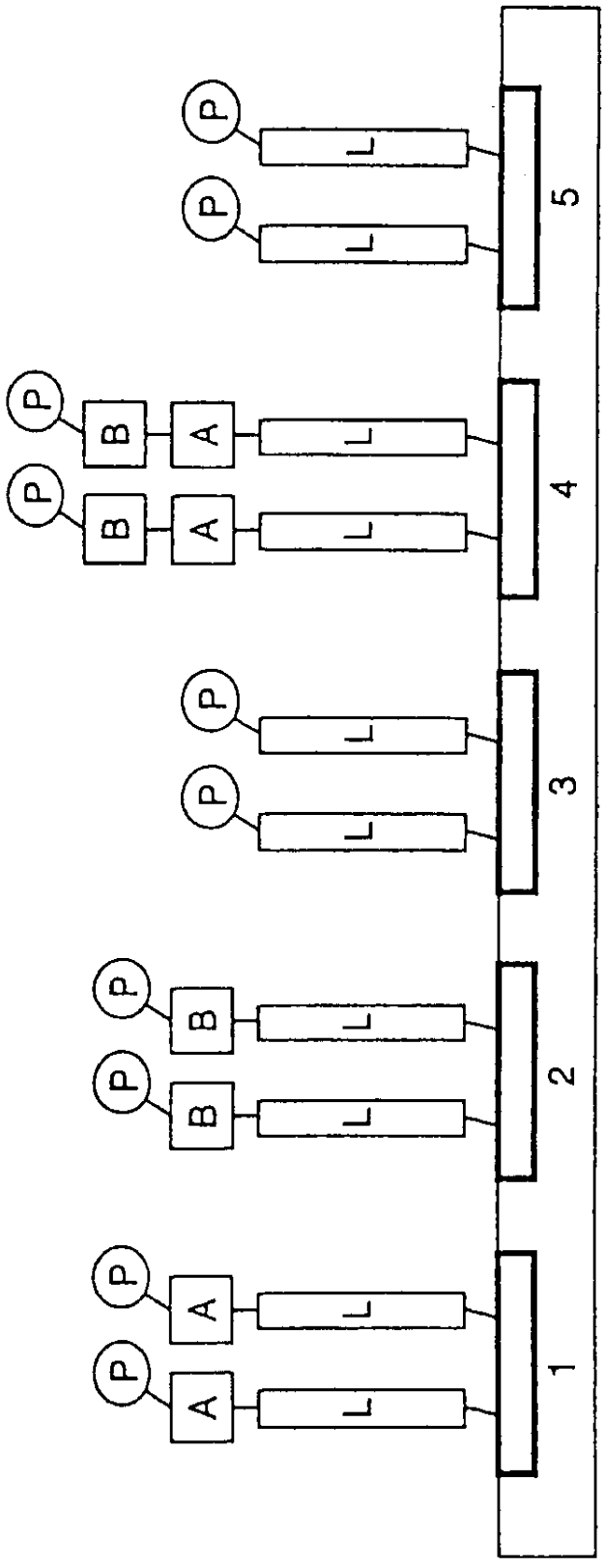


FIG. 4b

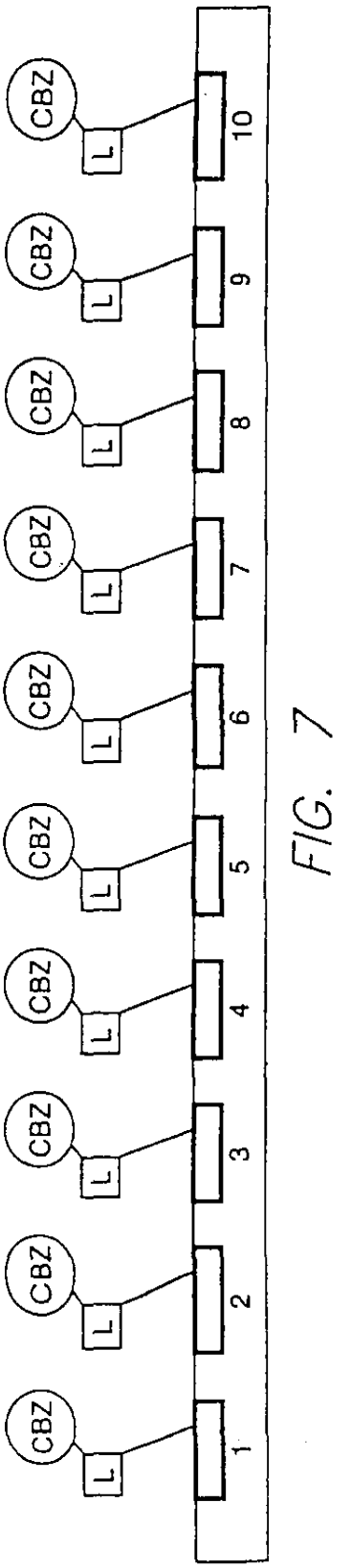


FIG. 7

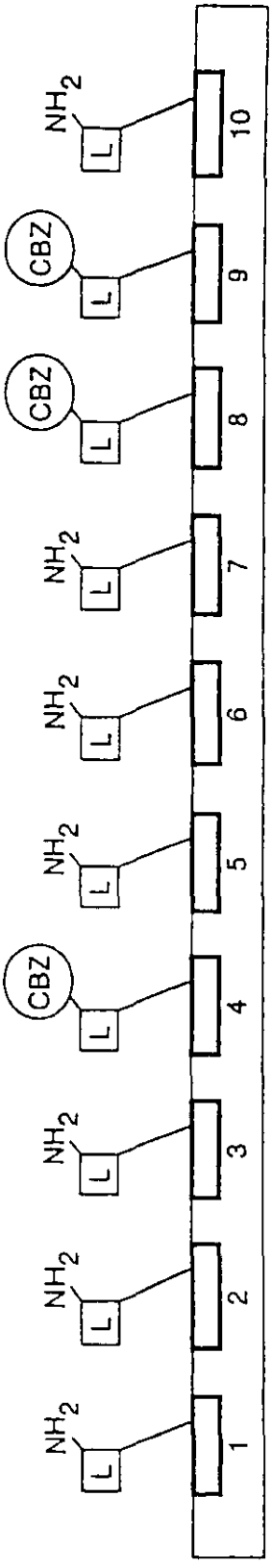


FIG. 8

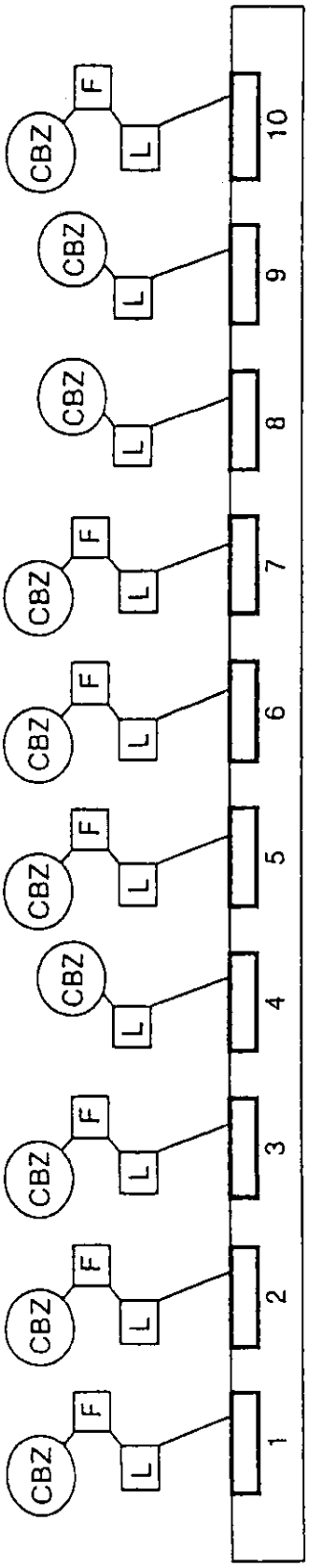


FIG. 9

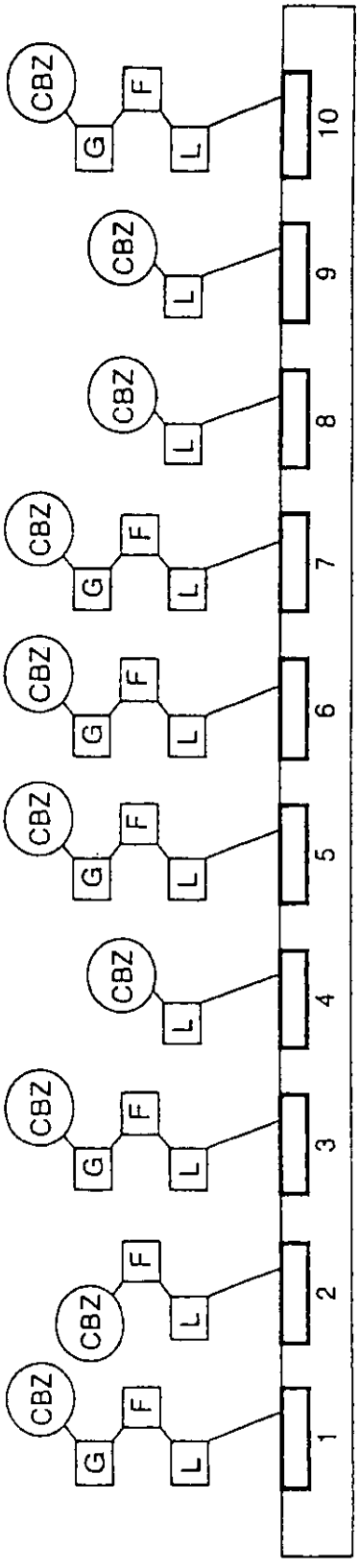
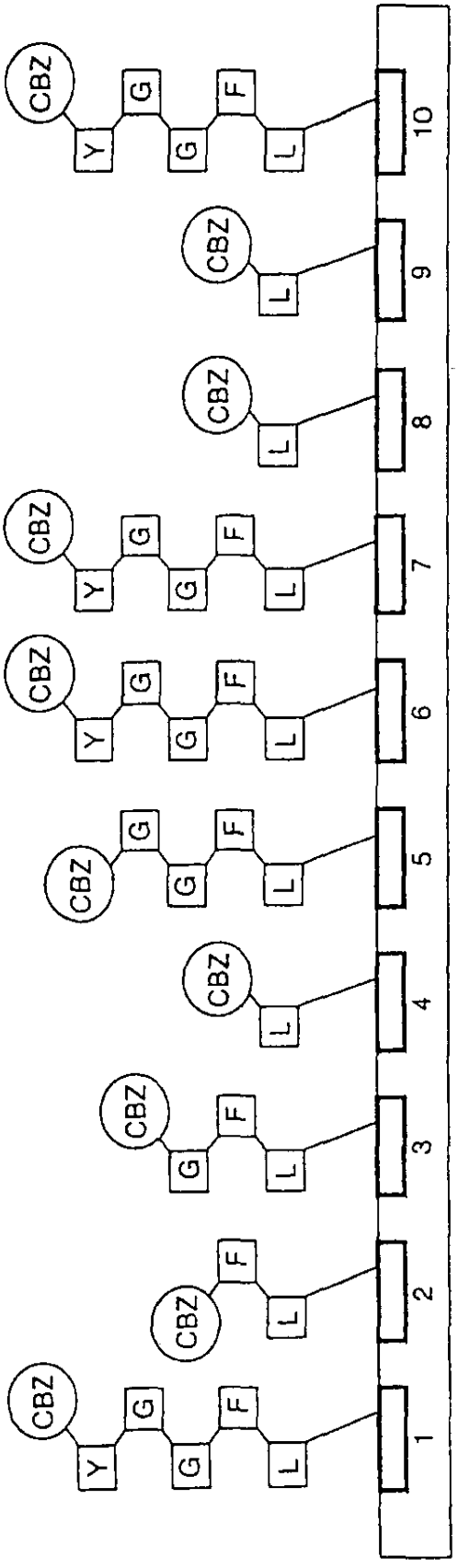


FIG. 10


CBZ
Y
G
G
F
L
10
CBZ
L
9
CBZ
L
8
CBZ
Y
G
G
F
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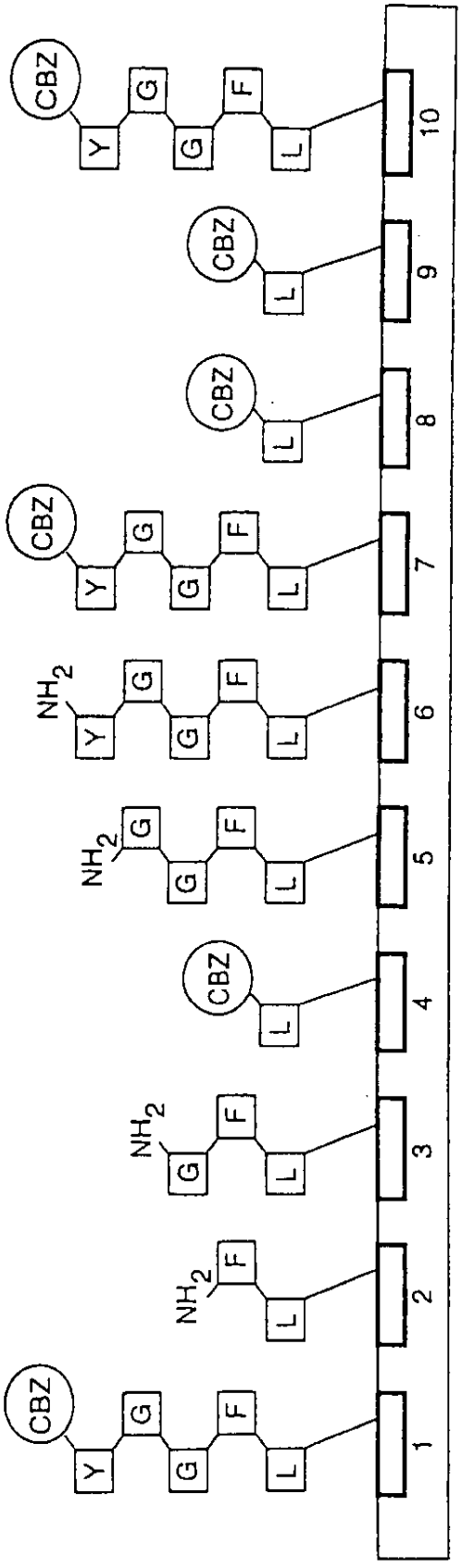
7
CBZ
Y
G
G
F
L
6
CBZ
G
G
F
L
5
CBZ
L
4
CBZ
G
F
L
3
CBZ
F
L
2
CBZ
Y
G
G
F
L
1

FIG. 11


CBZ
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G
G
F
L
10
CBZ
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9
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L
8
CBZ
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G
G
F
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NH2
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G
G
F
L
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NH2
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G
F
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5
CBZ
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2
CBZ
Y
G
G
F
L
1

FIG. 12

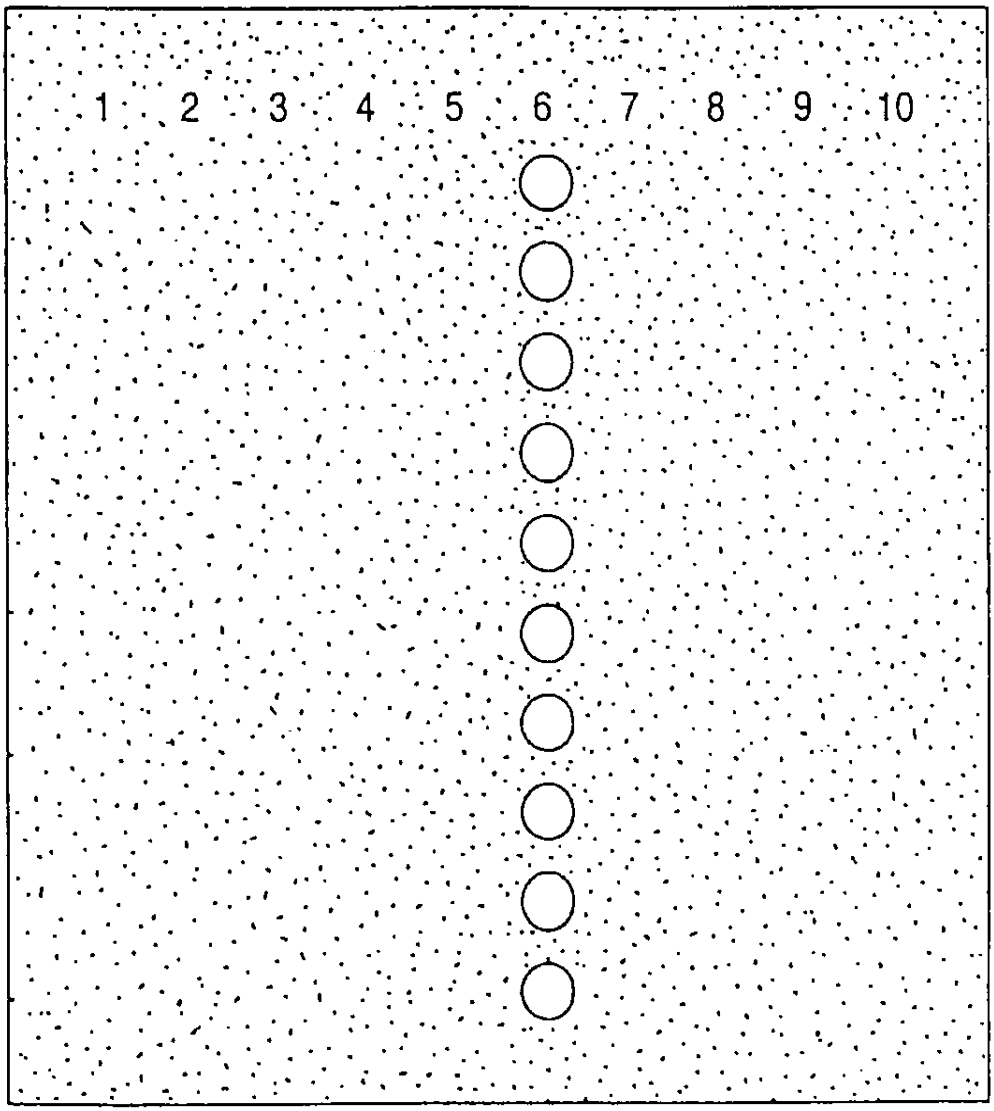


FIG. 13

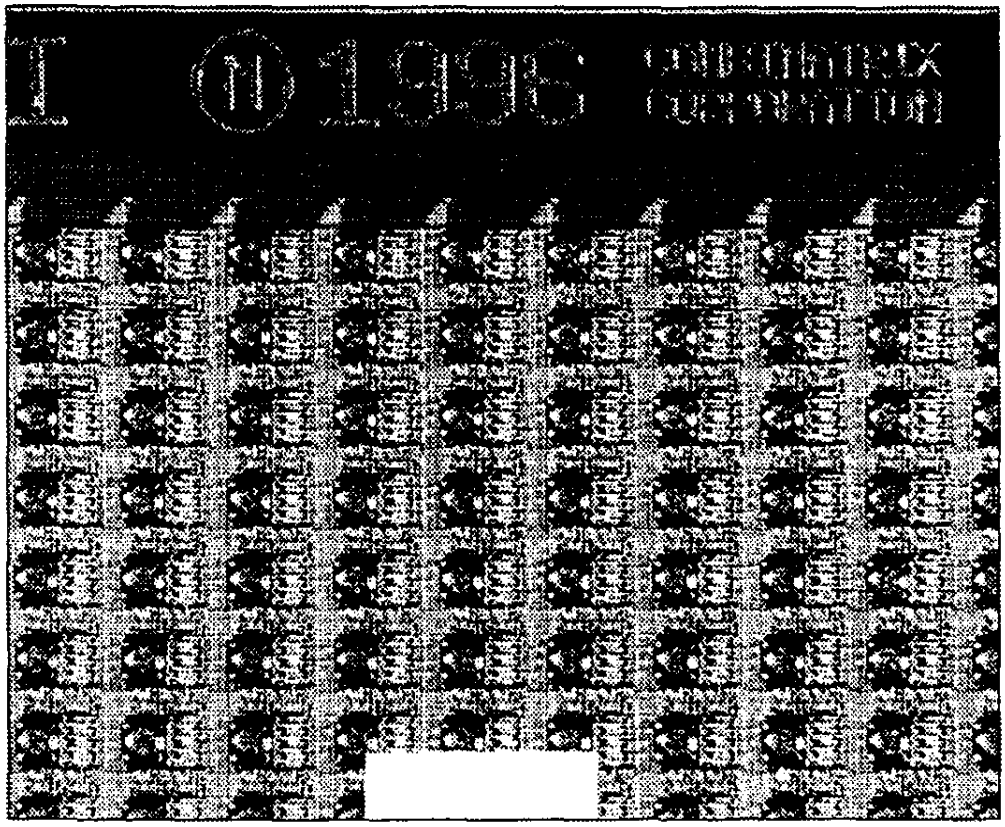


FIG. 14

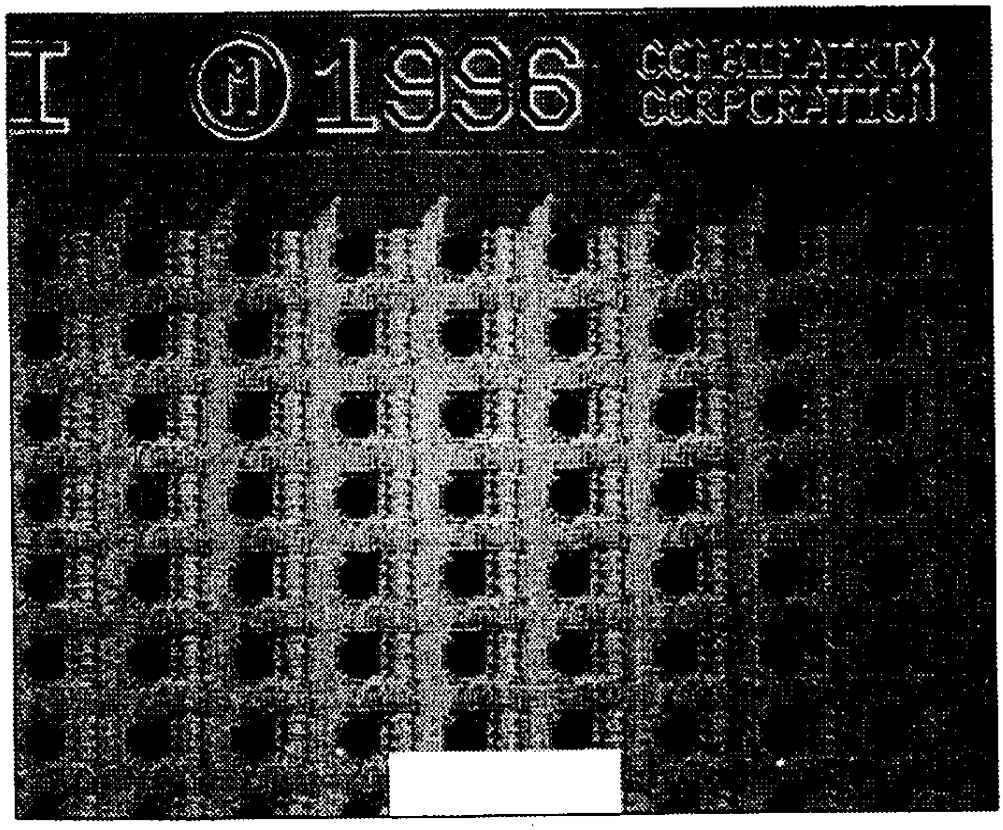


FIG. 15

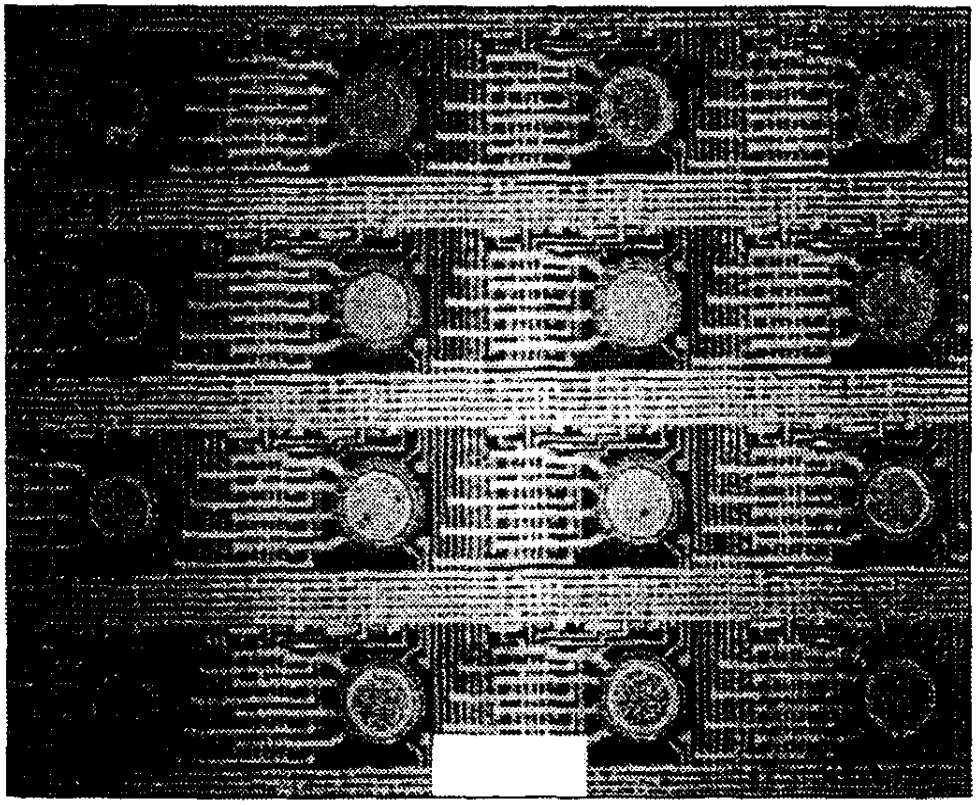


FIG. 16

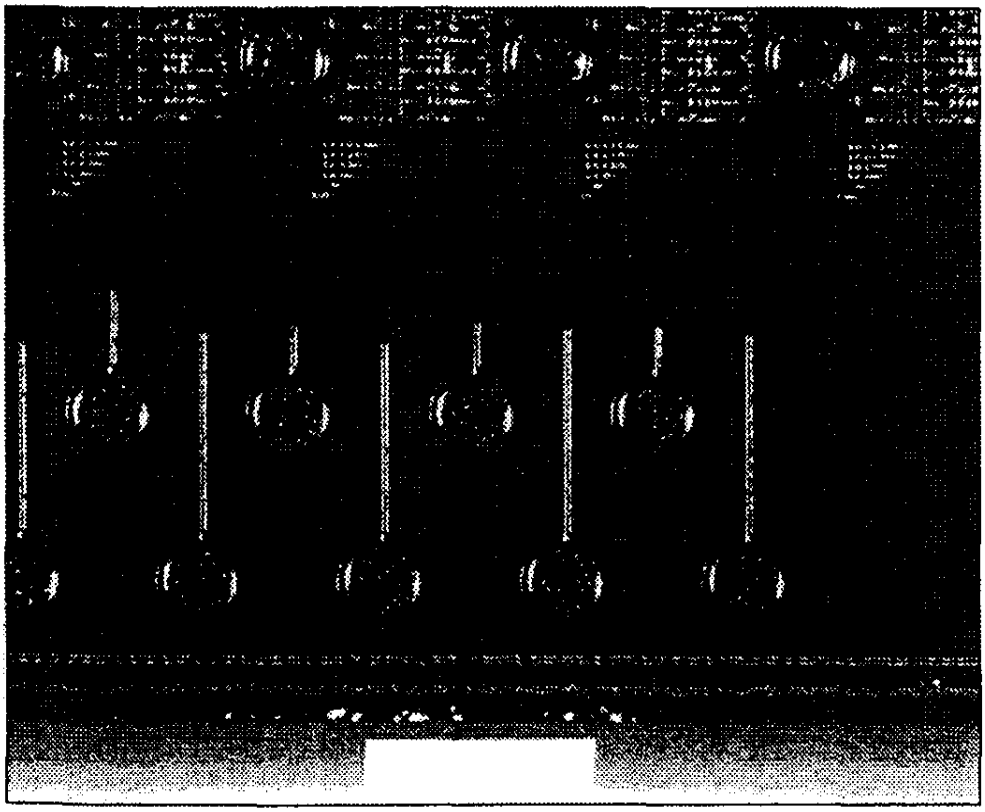


FIG. 17

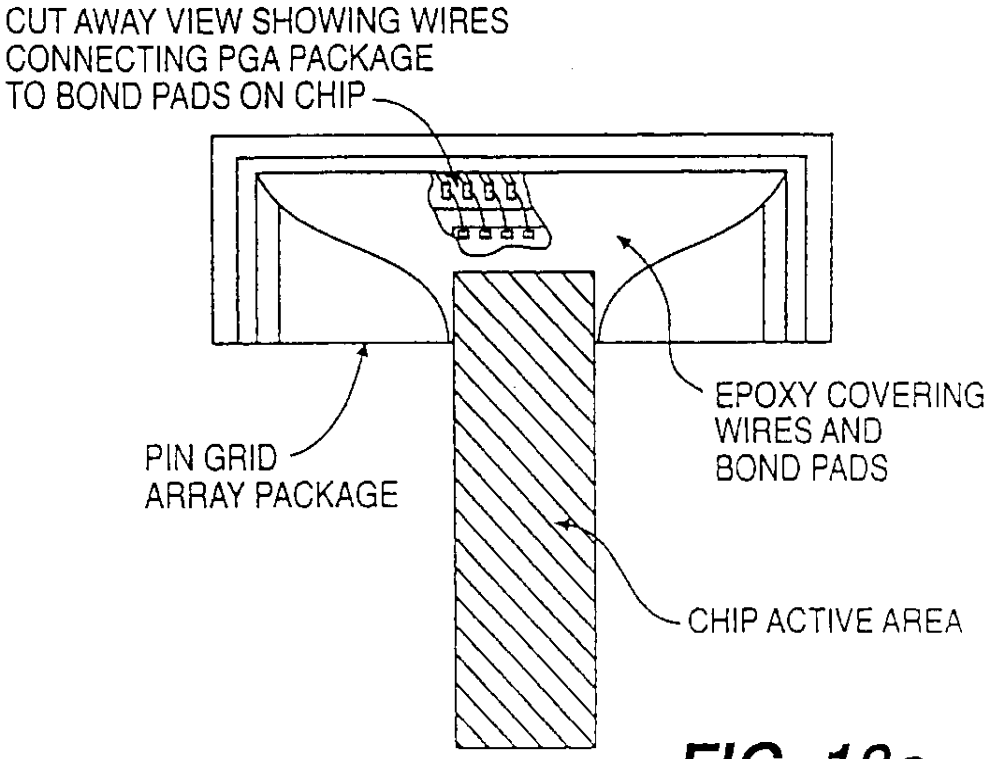


FIG. 18a

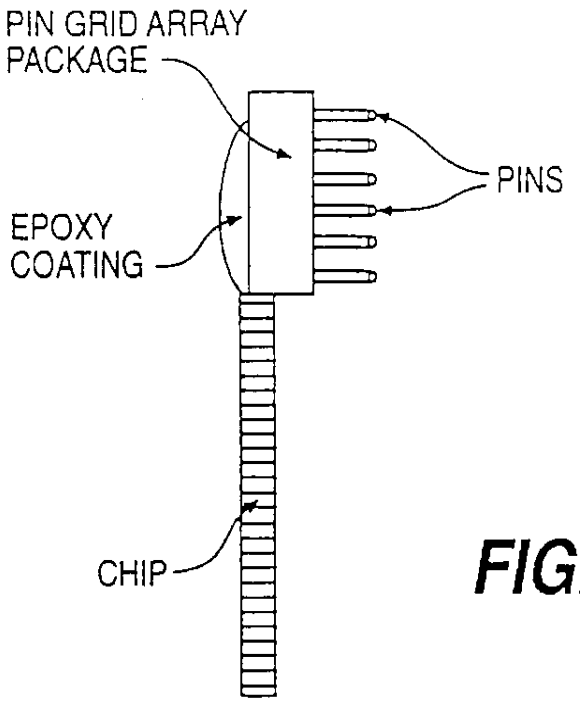


FIG. 18b

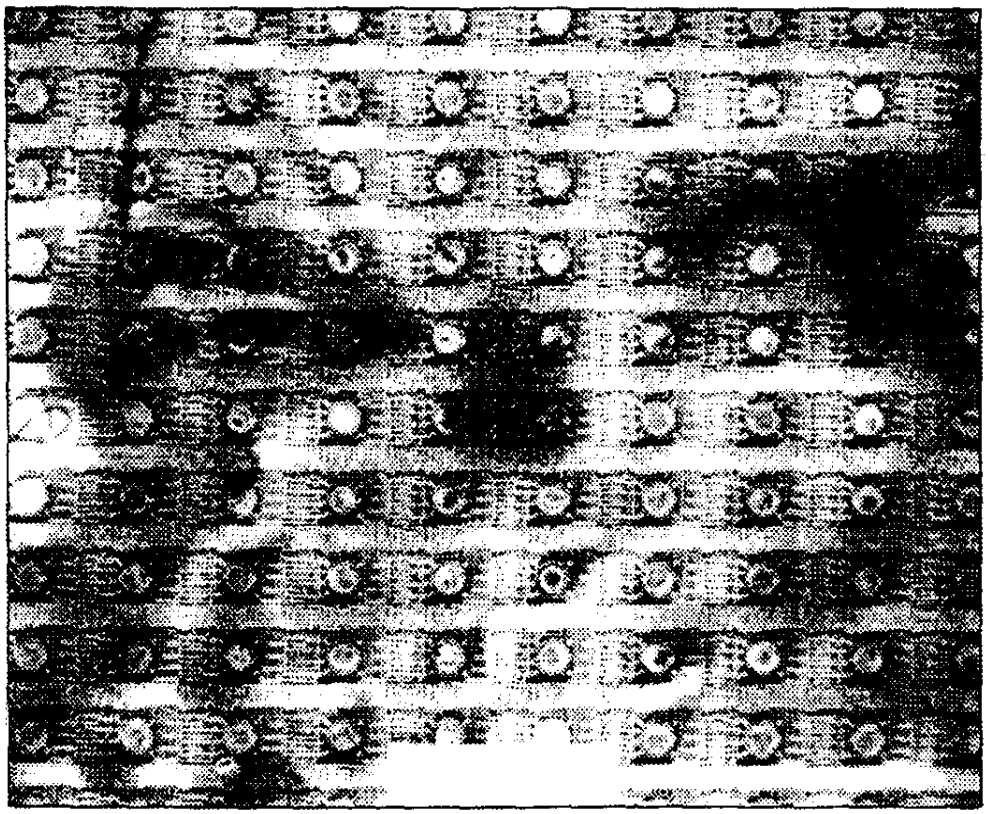


FIG. 19a

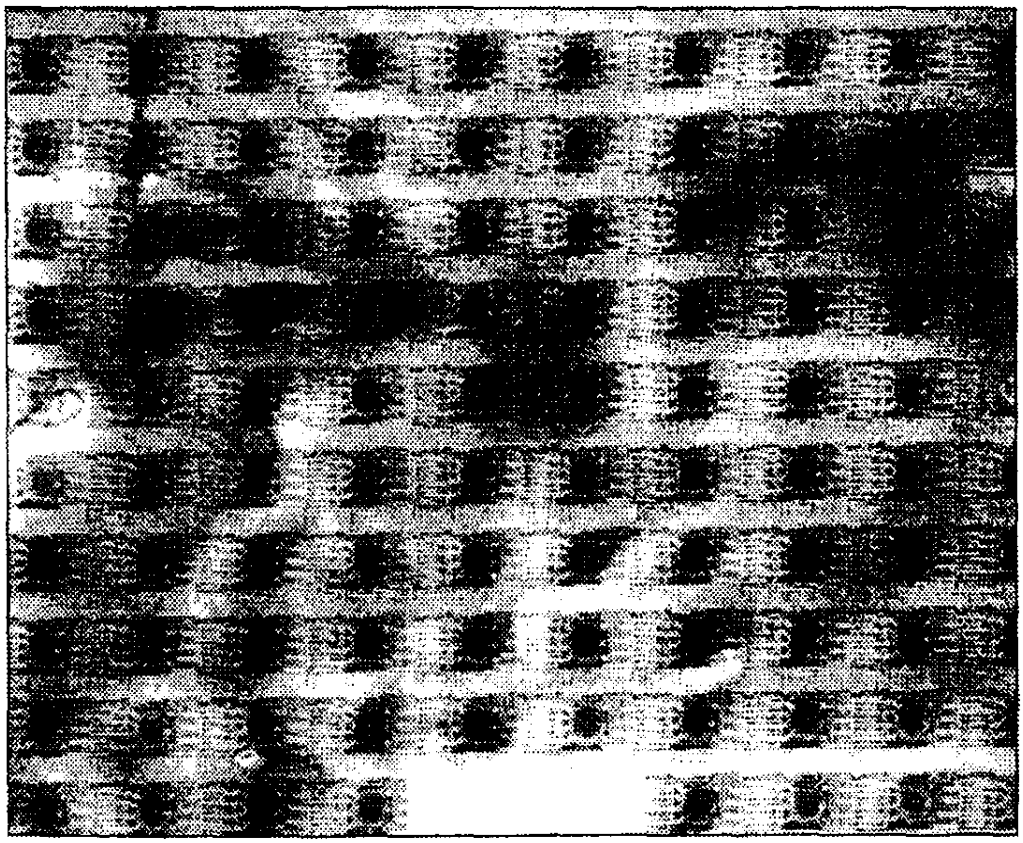


FIG. 19b

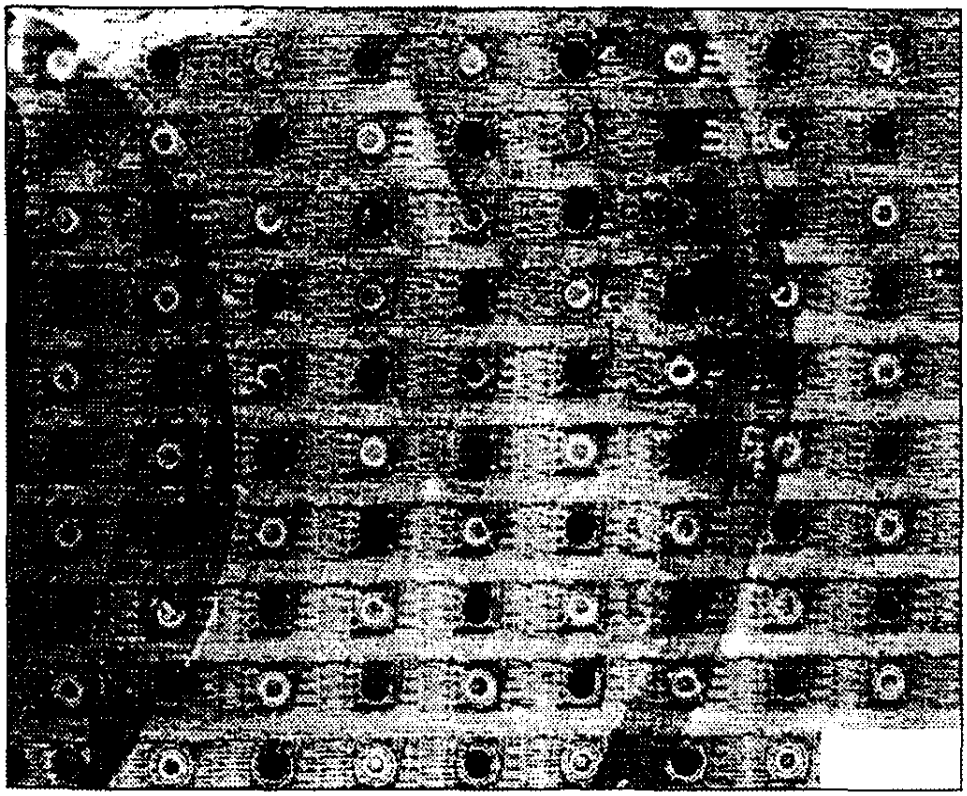


FIG. 20

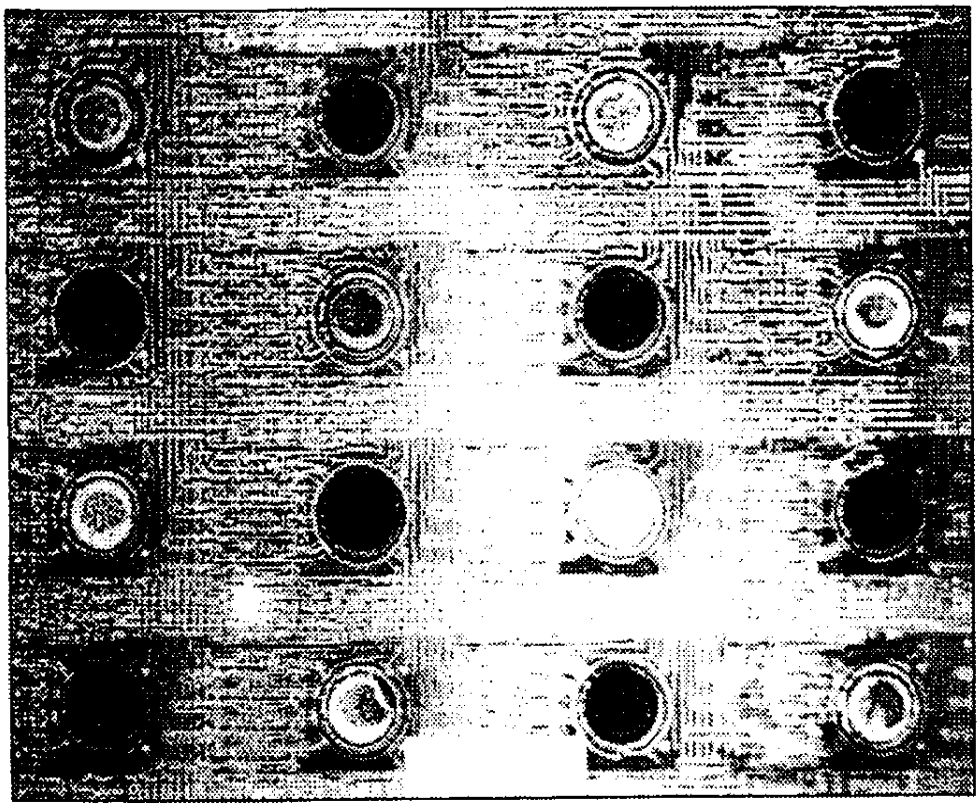


FIG. 21

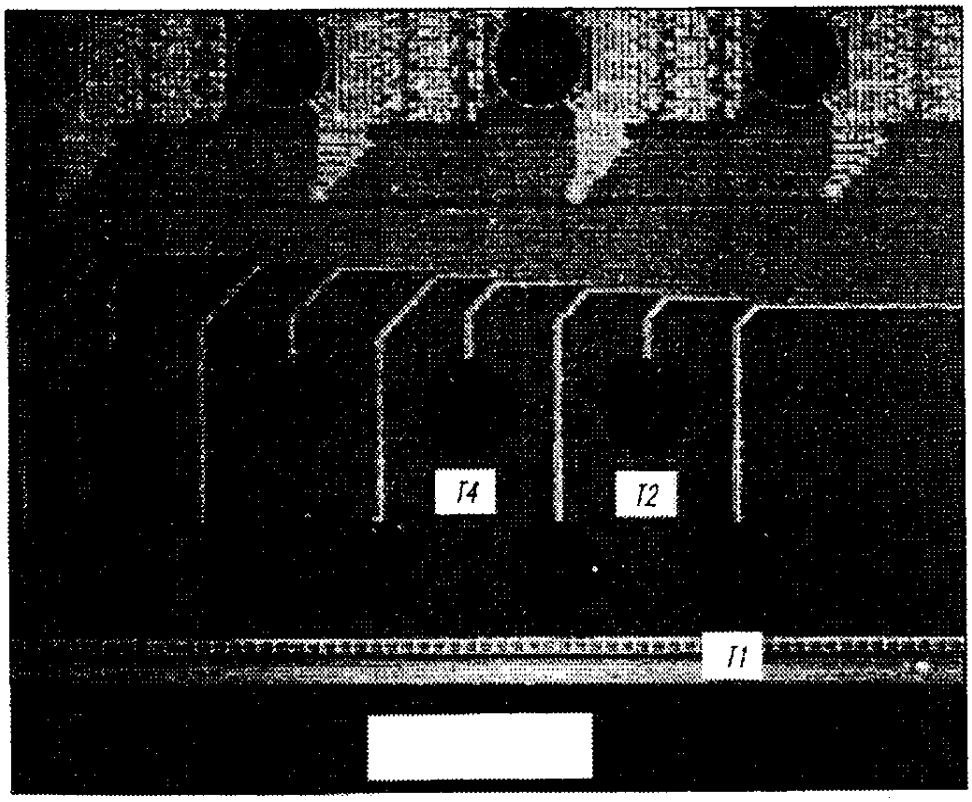


FIG. 22

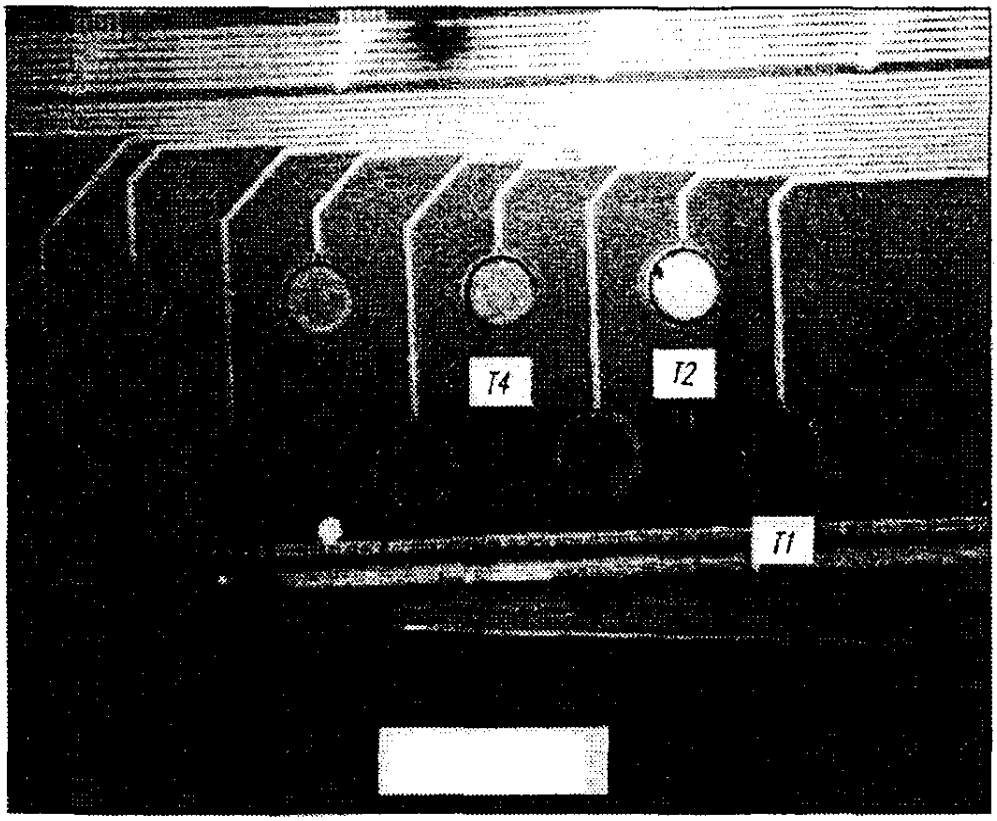


FIG. 23

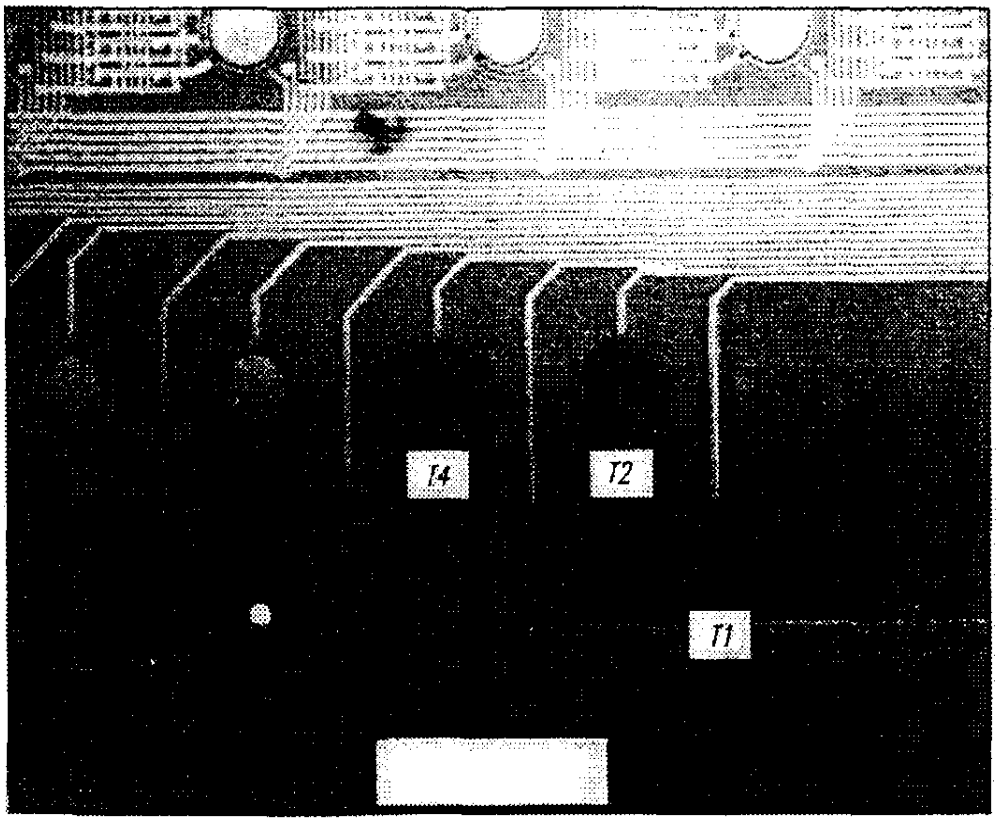


FIG. 24

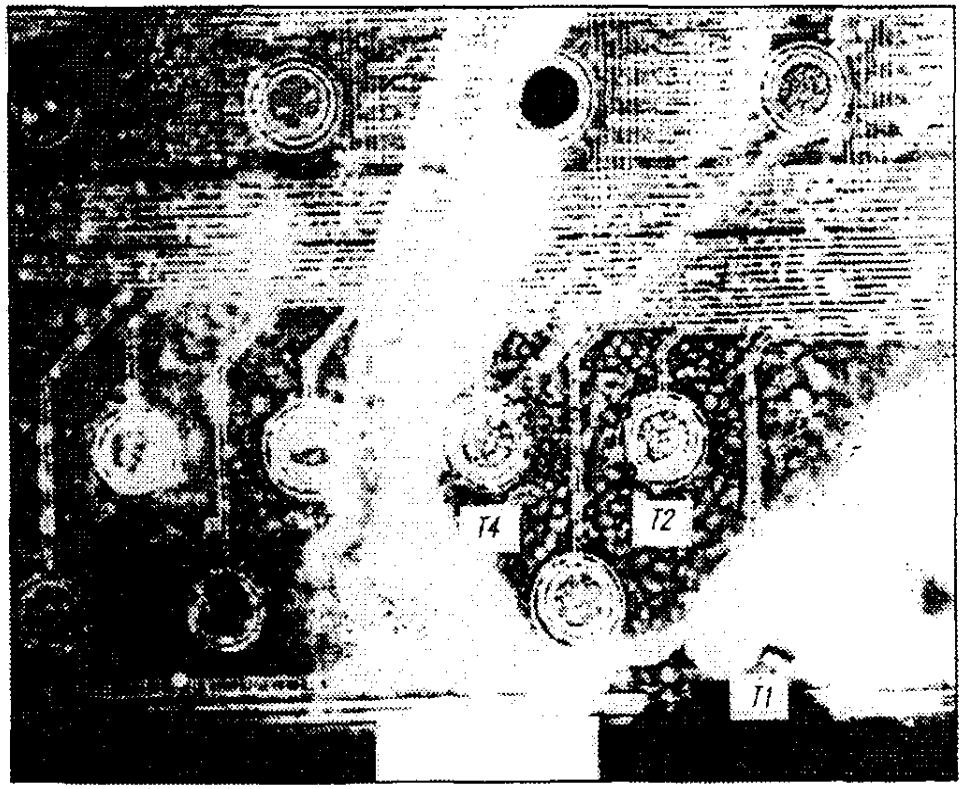


FIG. 25a

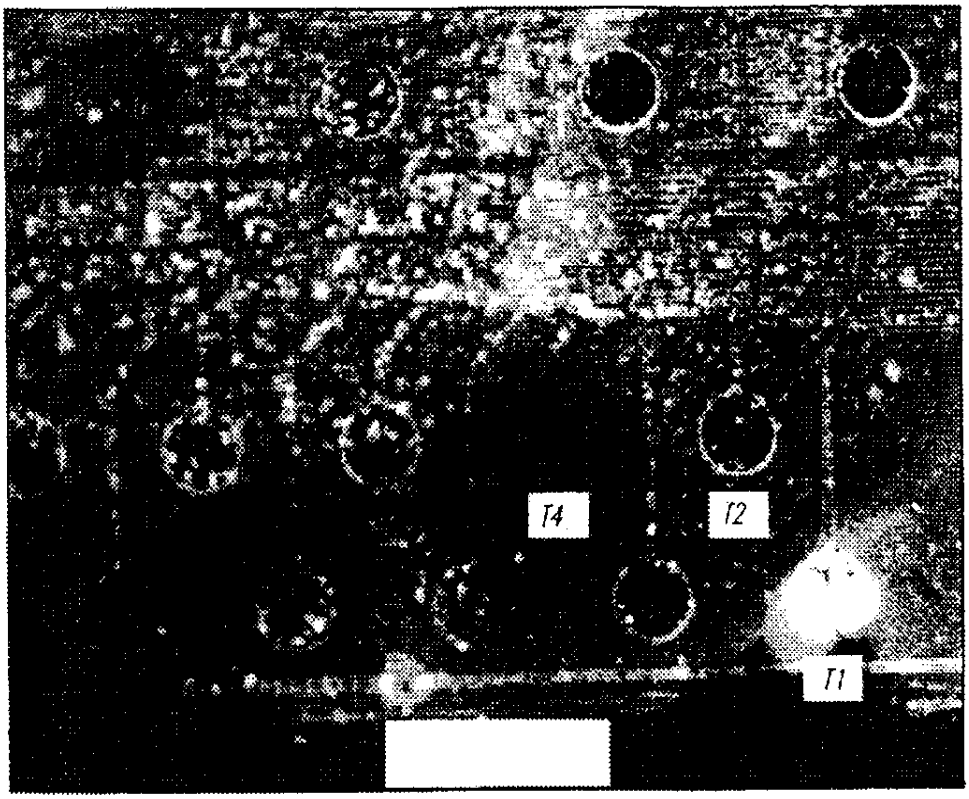


FIG. 25b

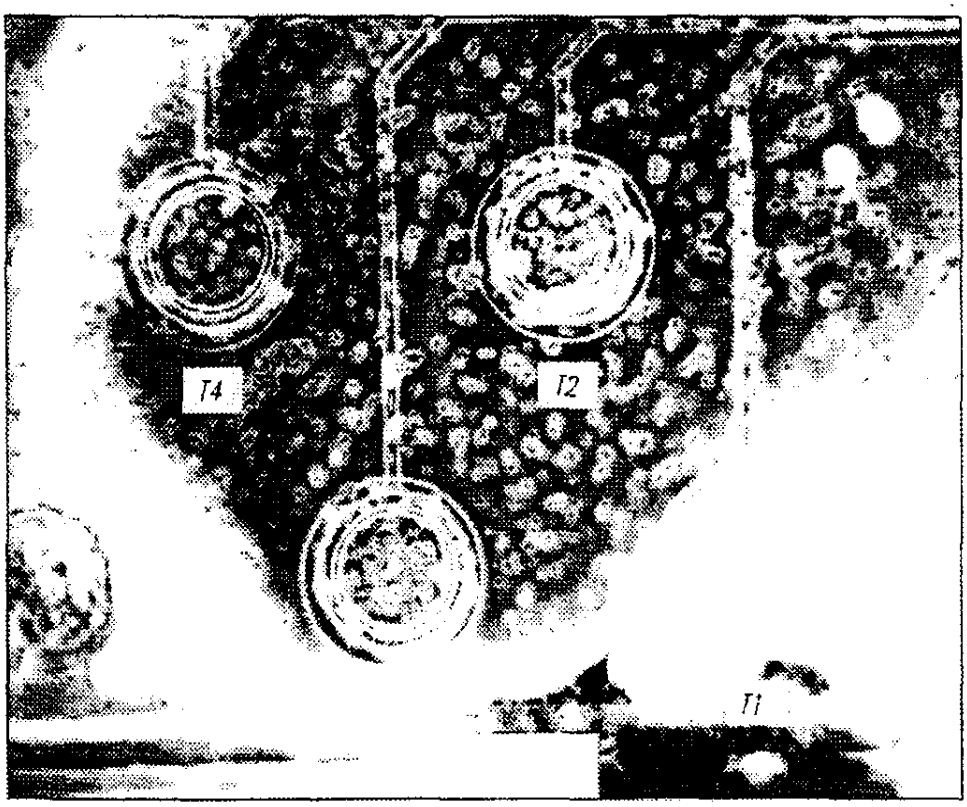


FIG. 26a

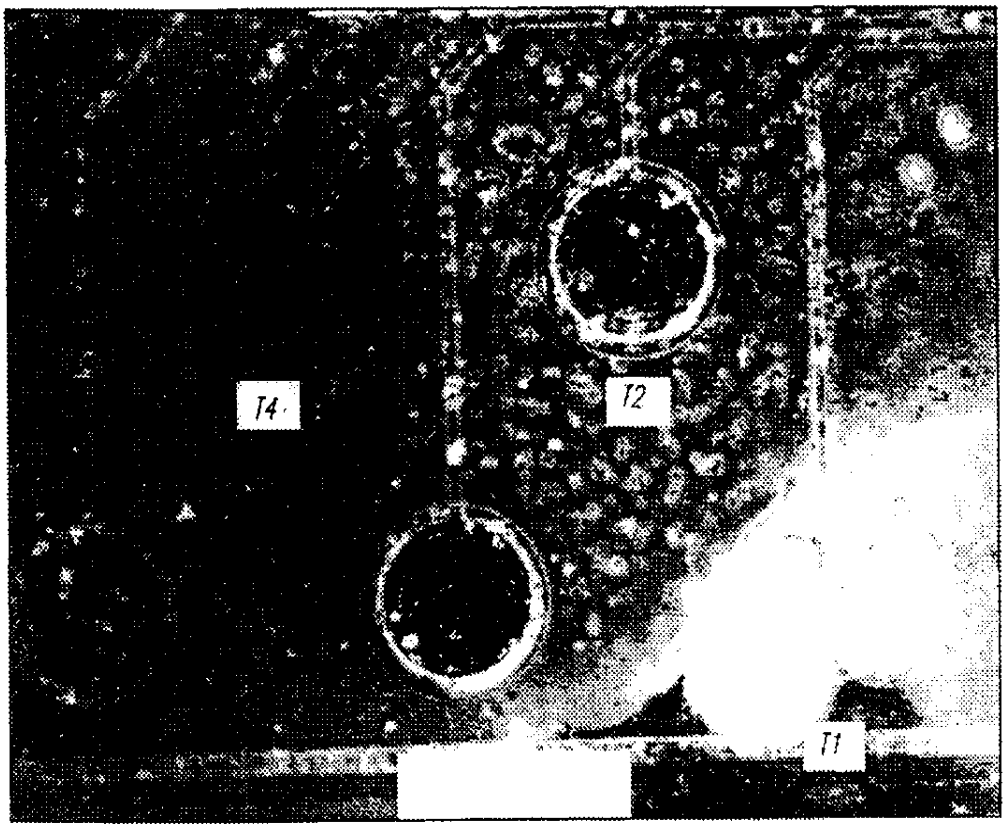


FIG. 26b

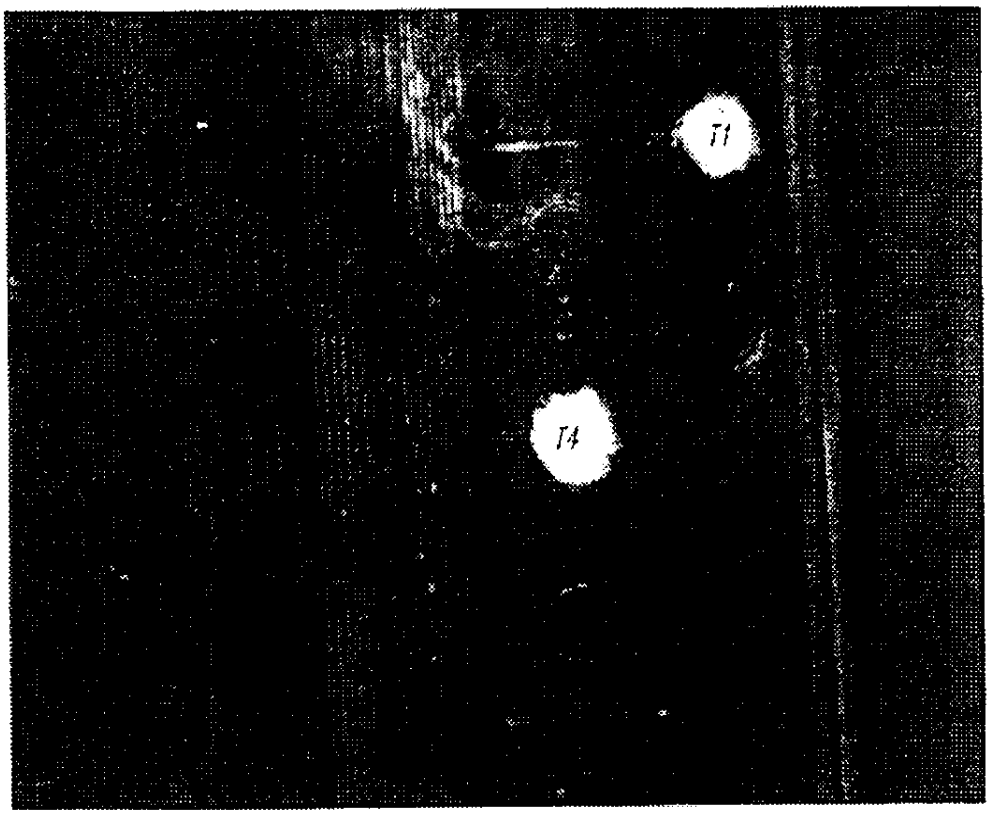


FIG. 27

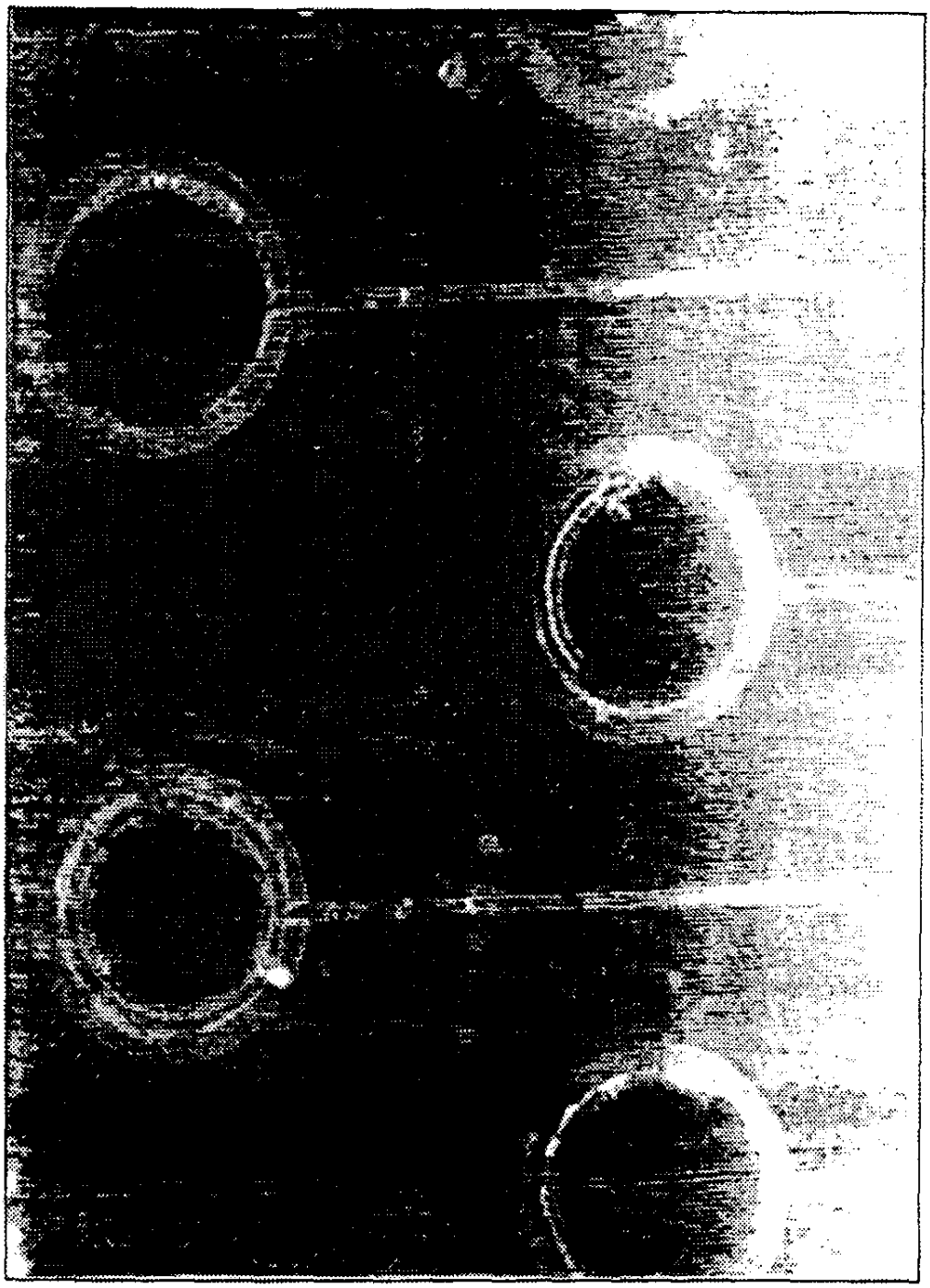


FIG. 28

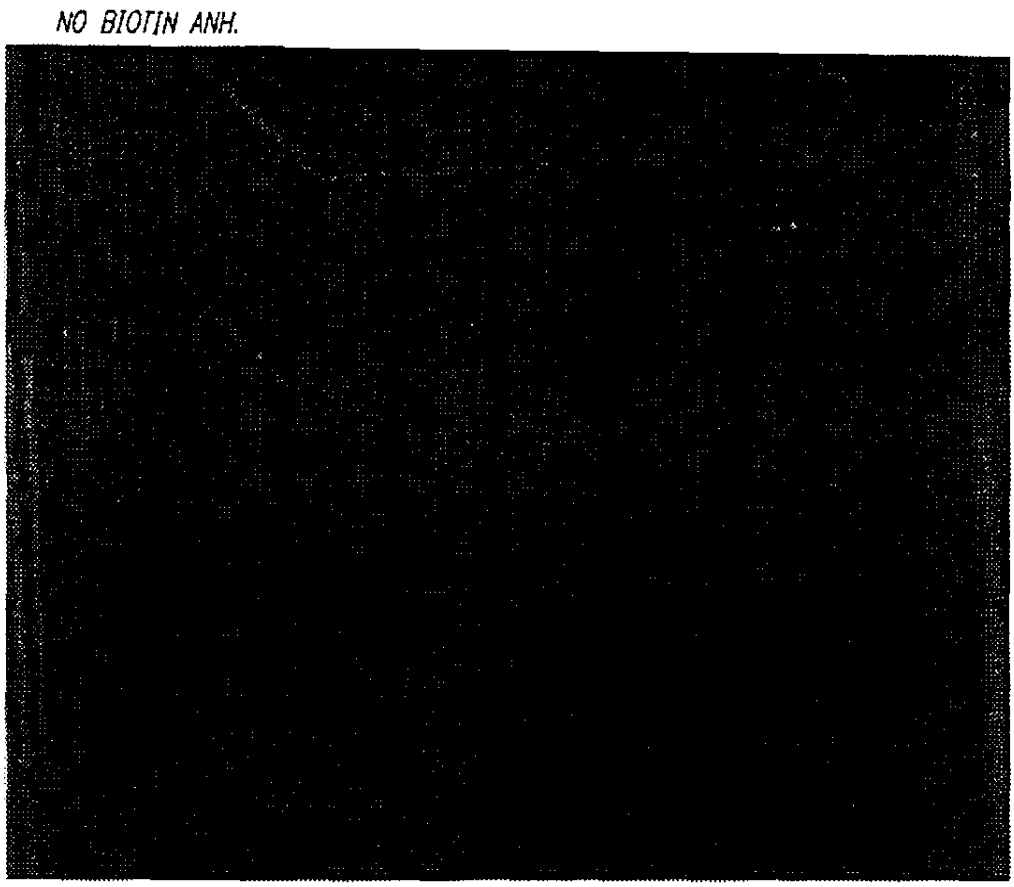


FIG. 29

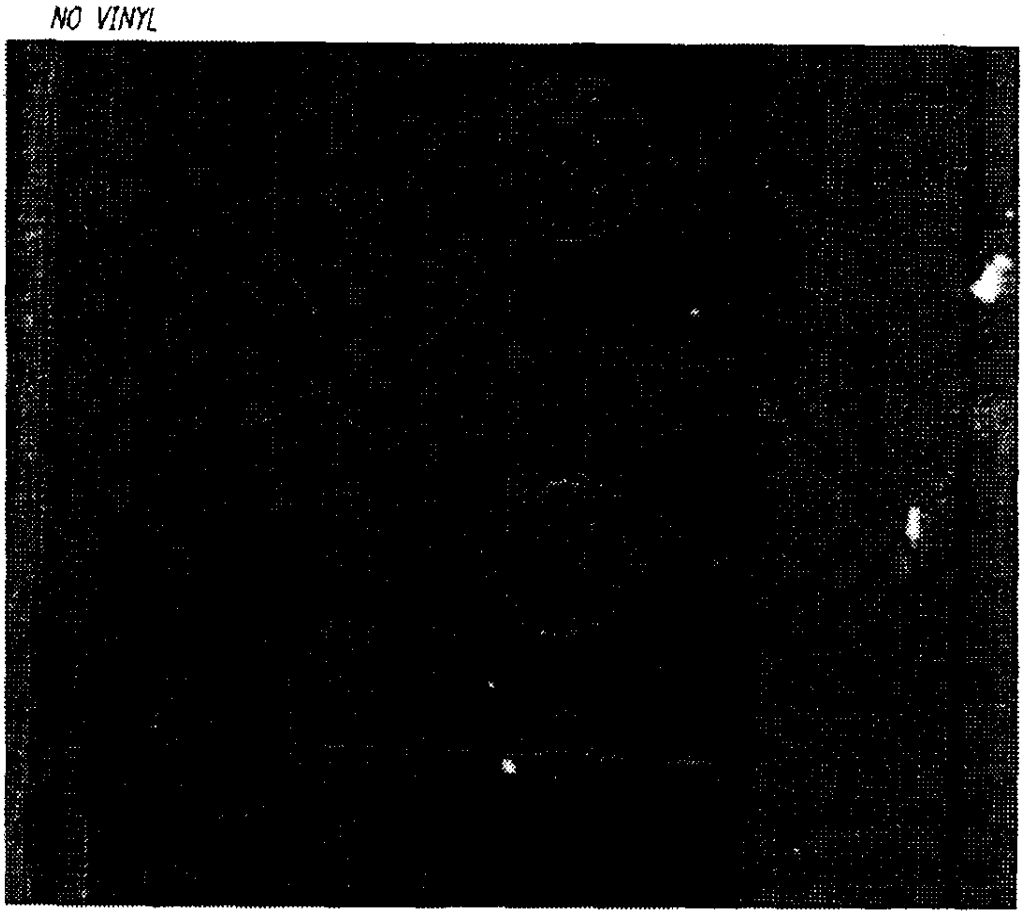
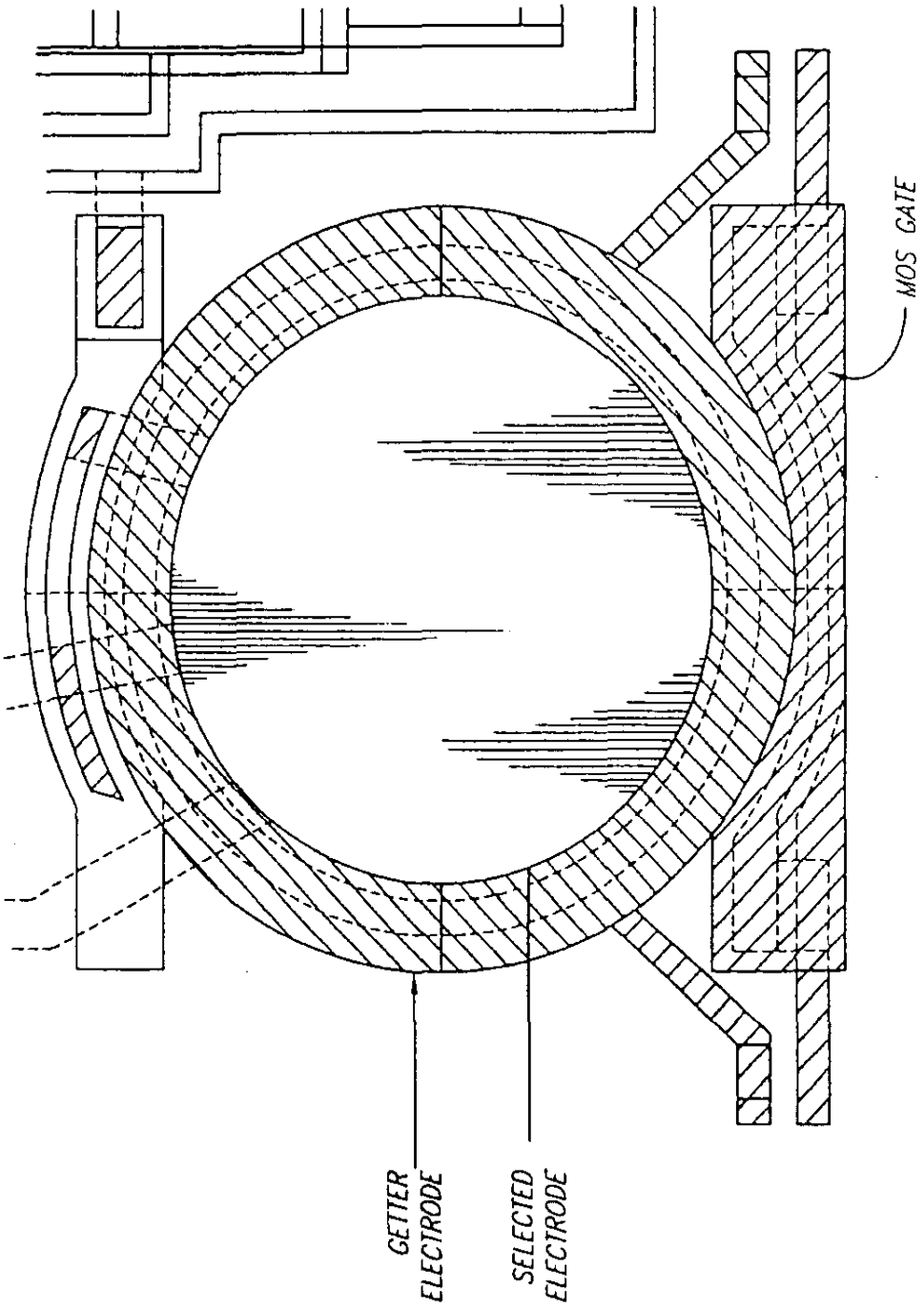


FIG. 30

FIG. 31



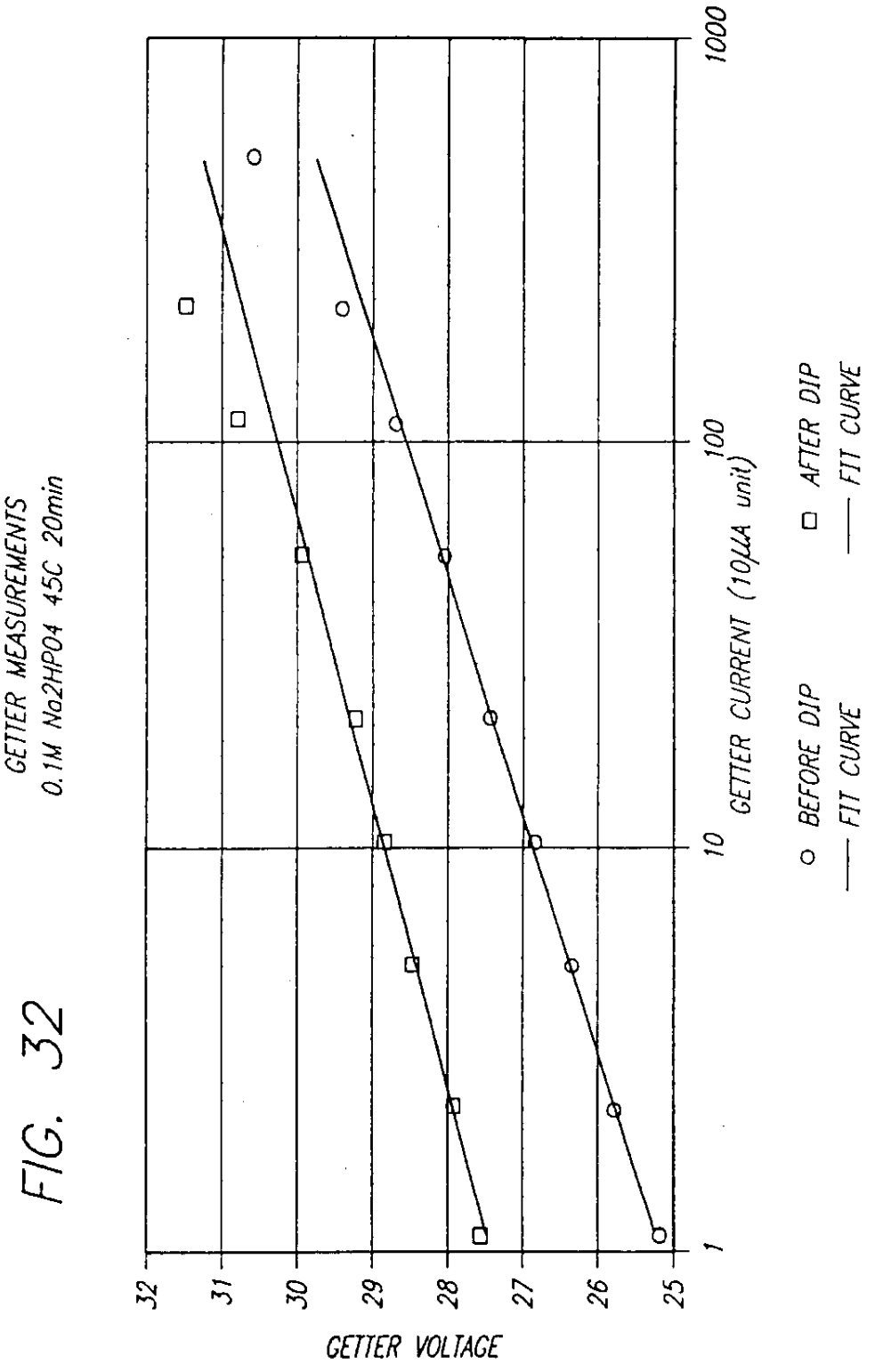

GETTER MEASUREMENTS
0.1M No2HP04 45C 20min
1000
100
10
1
GETTER CURRENT (10μA unit)
32
31
30
29
28
27
26
25
GETTER VOLTAGE
AFTER DIP
FIT CURVE
BEFORE DIP
FIT CURVE

FIG. 32

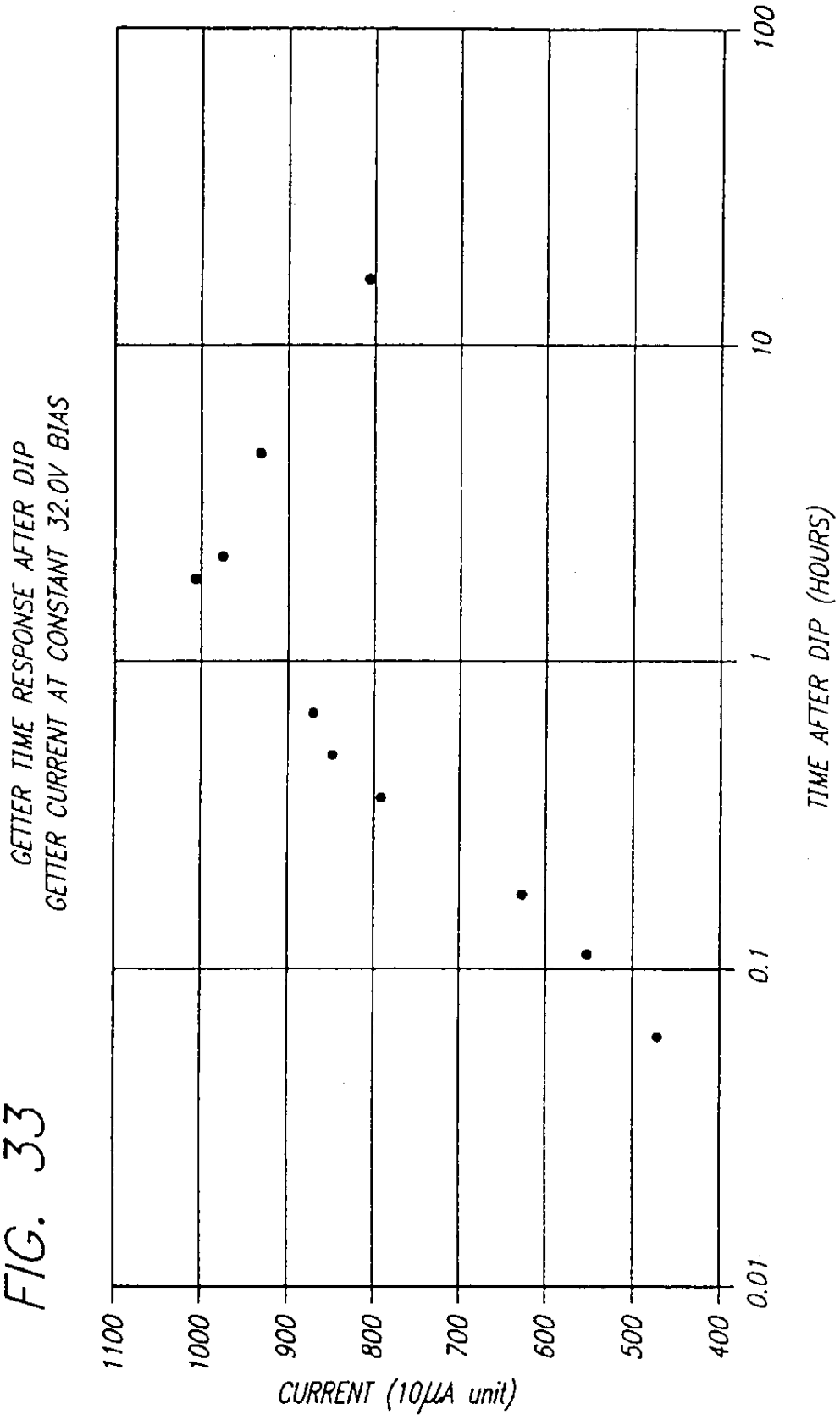

GETTER TIME RESPONSE AFTER DIP
GETTER CURRENT AT CONSTANT 32.0V BIAS
TIME AFTER DIP (HOURS)
100
10
1
0.1
0.01
1100
1000
900
800
700
600
500
400
CURRENT (10μA unit)

FIG. 33

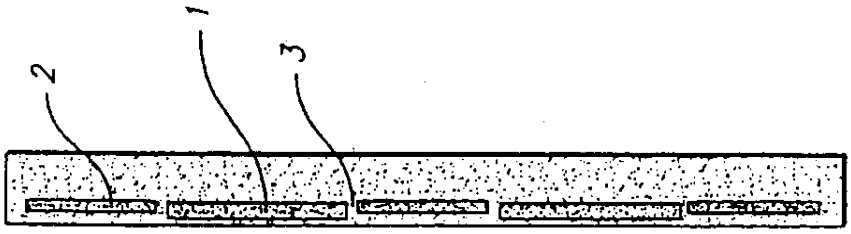


FIG. 34b

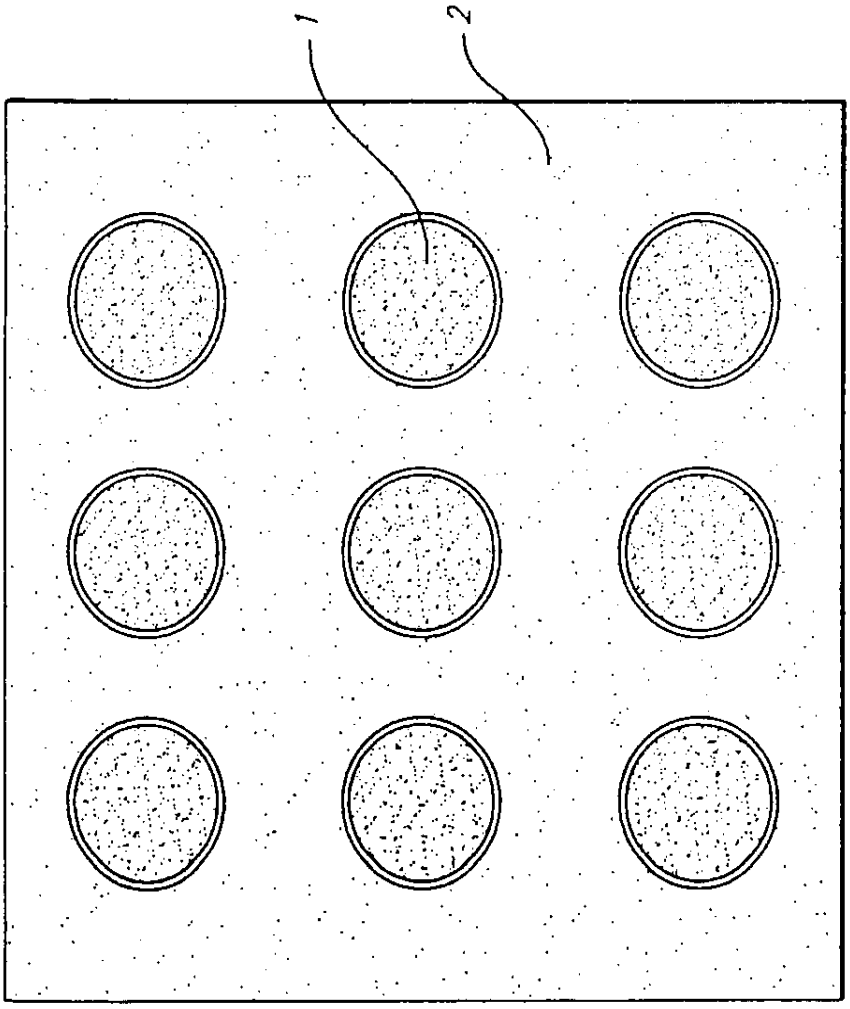


FIG. 34a

FIG. 36a

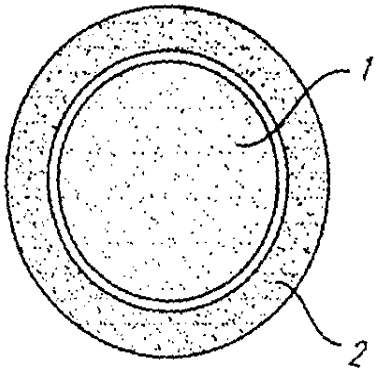


FIG. 35a

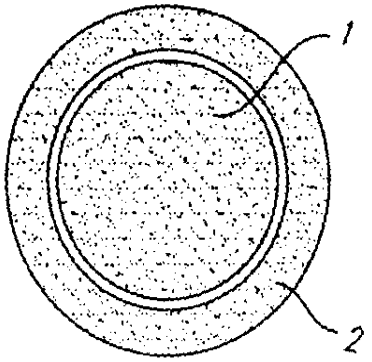


FIG. 36b

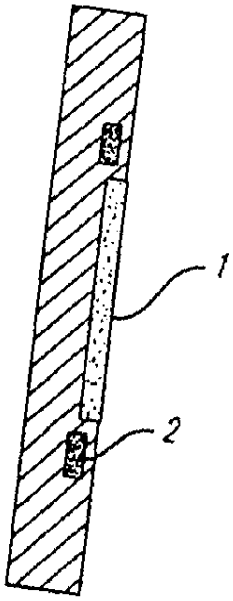
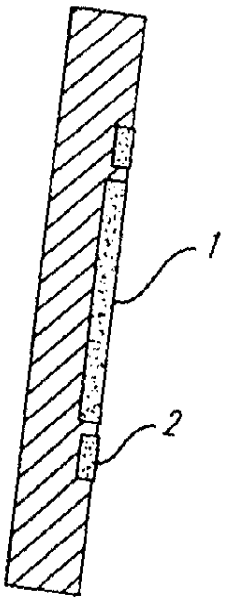


FIG. 35b



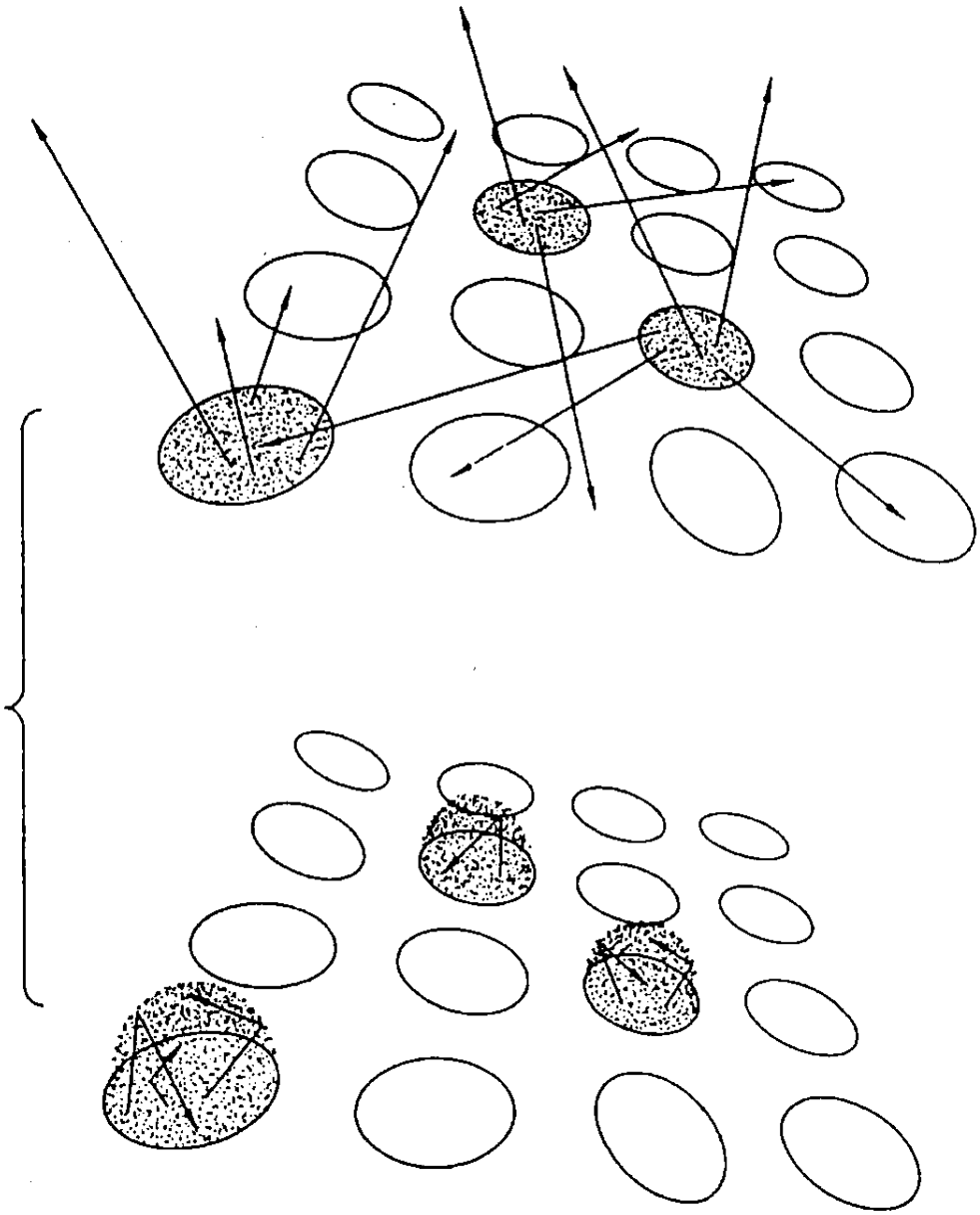


FIG. 37

ELECTROCHEMICAL SOLID PHASE SYNTHESIS

FIELD OF THE INVENTION

The present invention is directed to the synthesis and placement of materials at select locations on a substrate. In particular, the present invention is directed to a method for providing separate sequences of chemical monomers at select locations on a substrate.

The present invention may be applied in the field of, but is not limited to, the preparation of peptide, oligomer, polymer, oligosaccharide, nucleic acid, ribonucleic acid, porphyrin, and drug congeners. In particular, the present invention may be used as a method to create sources of chemical diversity for use in screening for biological activity, for example, for use in the rapidly developing field of combinatorial chemistry.

BACKGROUND OF THE INVENTION

There are many known assays for measuring the binding capabilities of known target molecules and the various molecules known to bind selectively to target molecules, i.e., ligands. The information that may be gained from such experiments often is limited by the number and type of ligands that are available. Continuing research is focused on the discovery of new ligands. Novel ligands are sometimes discovered by chance, or by application of techniques for the elucidation of molecular structure, or by systematic analysis of the relationships between molecular structure and binding activity.

Small peptide molecules are useful model systems for exploring the relationship between structure and function in biology. A peptide is a sequence of amino acids. For example, the twenty naturally occurring amino acids can be condensed into polymeric molecules. These polymeric molecules form a large variety of three-dimensional spatial and electronic structures. Each structure arises from a particular amino acid sequence and solvent condition. The number of possible hexapeptides of the twenty naturally occurring amino acids, for example, is 206, or 64 million different peptides. As shown by epitope studies, the small peptide molecules are useful in target-binding studies, and sequences as short as a few amino acids are recognized with high specificity by some antibodies.

The process of discovering ligands with desirable patterns of specificity for targets of biological importance is central to many contemporary approaches to drug discovery. These approaches, based on structure-activity relationships, involve rational design of ligands and large scale screening of families of potential ligands. Often, a combination of approaches is used. The ligands are often, but not exclusively, small peptide molecules.

Yet methods of preparing large numbers of different ligands have been painstakingly slow and prohibitively expensive when used at a scale sufficient to permit effective rational or random screening. For example, the well-known "Merrifield" method (*J. Am. Chem. Soc.* (1963) 85:2149–2154), which is incorporated herein by reference, has been used to synthesize peptides on solid supports. In the Merrifield method, an amino acid is bound covalently to a support made of an insoluble polymer. Another amino acid with an alpha protected group is reacted with the covalently bonded amino acid to form a dipeptide. After washing, the protective group is removed and a third amino acid with an alpha protective group is added to the dipeptide. This process is continued until a peptide of a desired length and

sequence is obtained. Using the Merrifield method, synthesis of more than a handful of peptide sequences in a day is not technically feasible or economically practical.

To synthesize larger numbers of polymer sequences, it has been proposed to use a series of reaction vessels for polymer synthesis. For example, a tubular reactor system may be used to synthesize a linear polymer on a solid phase support by automated sequential addition of reagents. This method, however, also does not enable the synthesis of a sufficiently large number of polymer sequences for effective and economical screening.

Another method of preparing a plurality of polymer sequences uses a porous container enclosing a known quantity of reactive particles, larger in size than pores of the container. The particles in the containers may be selectively reacted with desired materials to synthesize desired sequences of product molecules. However, as with the other methods known in the art, this method is not practical for the synthesis of a sufficient variety of polypeptides for effective screening.

Other techniques have also been described and attempted. Several of these methods include synthesis of peptides on 96 plastic pins that fit the format of standard microtiter plates. Unfortunately, while these techniques have been somewhat useful, substantial problems remain. For example, methods using standard microtiter plates continue to be limited in the diversity of sequences that can be synthesized and screened. Although it is recognized that using microtiter plates produces essentially pure polymers because each polymer is synthesized in an isolated well of the microtiter plate, the number of polymers that can be produced in any given time is limited by the number of wells in a microtiter plate, i.e., 96. Moreover, the equipment needed for synthesis in the microtiter plates is large. Because of this limitation, use of microtiter plates requires a large amount of space to produce a relatively small number of peptides.

One attempt at synthesizing a large number of diverse arrays of polypeptides and polymers in a smaller space is found in U.S. Pat. No. 5,143,854 granted to Pirrung et al. (1992). This patent describes the use of photolithographic techniques for the solid phase synthesis of arrays of polypeptides and polymers. The disclosed technique uses "photomasks" and photolabile protecting groups for protecting the underlying functional group. Each step of the process requires the use of a different photomask to control which regions are exposed to light and thus deprotected. The necessity of having to fabricate a new set of photomasks for each array of chemical monomers results in a method that is extremely expensive and not well-suited to automation. Moreover, this method is tedious and time consuming because each step of the synthesis requires the mechanical removal, replacement and realignment of a photomask. Thus, synthesizing a large number of libraries of polymers with the Pirrung method is an inefficient and uneconomical process.

Another drawback of the Pirrung method is that the photolabile protecting groups used cannot be removed as effectively as conventional acid or base labile protecting groups can be removed and are plagued by contamination due to undesired side reactions. Consequently, using Pirrung's method, the purity of the chemical array is often compromised due to incomplete removal of the protecting groups and subsequent failure of the underlying functional groups to react with the desired monomer, as well as contamination from undesired side reactions.

Another attempt to synthesize large numbers of polymers is disclosed by Southern in International patent application

WO 93/22480, published Nov. 11, 1993. Southern describes a method for synthesizing polymers at selected sites by electrochemically modifying a surface; this method involves providing an electrolyte overlaying the surface and an array of electrodes adjacent to the surface. In each step of Southern's synthesis process, an array of electrodes is mechanically placed adjacent the points of synthesis, and a voltage is applied that is sufficient to produce electrochemical reagents at the electrode. The electrochemical reagents are deposited on the surface themselves or are allowed to react with another species, found either in the electrolyte or on the surface, in order to deposit or to modify a substance at the desired points of synthesis. The array of electrodes is then mechanically removed and the surface is subsequently contacted with selected monomers. For subsequent reactions, the array of electrodes is again mechanically placed adjacent the surface and a subsequent set of selected electrodes activated.

This method requires that a large amount of control be exercised over the distance that exists between the electrode array and the surface where synthesis occurs. Control over the distance between the electrodes and the surface for modification is required to ensure precise alignment between the electrodes and the points of synthesis and to limit the extent of diffusion of electrochemically generated reagents away from the desired points of synthesis. However, the inherent difficulty in positioning electrodes repeatedly and accurately within a few microns of the surface frequently results in the production of electrochemically generated reagents at undesirable synthesis points. Moreover, the diffusion of the electrochemically generated reagents from desired sites of reaction to undesired sites of reaction results in "chemical cross-talk" between synthesis sites. This cross-talk severely compromises the purity of the final product, as undesired binding reactions occur at unselected sites. The amount of cross-talk is further aggravated by the disruptions of surface tension that occur whenever the electrodes are moved, leading to convective mixing that causes increased movement of the electrochemically generated reagents. While Southern attempts to minimize the amount of cross-talk by applying a potential designed to counteract diffusion, as a practical matter, the electric fields Southern can generate are too low to prevent diffusion. When the potential is raised to increase the electric field, large quantities of undesired electrochemically generated reagents are produced. Hence, Southern is not a practical method for generating large numbers of pure polymers.

A more recent attempt to automate the synthesis of polymers is disclosed by Heller in International patent application WO 95/12808, published May 11, 1995. Heller describes a self-addressable, self-assembling microelectronic system that can carry out controlled multi-step reactions in microscopic environments, including biopolymer synthesis of oligonucleotides and peptides. The Heller method employs free field electrophoresis to transport analytes or reactants to selected micro-locations where they are effectively concentrated and reacted with the specific binding entities. Each micro-location of the Heller device has a derivatized surface for the covalent attachment of specific binding entities, which includes an attachment layer, a permeation layer, and an underlying direct current microelectrode. The presence of the permeation layer prevents any electrochemically generated reagents from interacting with or binding to either the points of synthesis or to reagents that are electrophoretically transported to each synthesis site. Thus, all synthesis is due to reagents that are electrophoretically transported to each site of synthesis.

The Heller method, however, is severely limited by the use of electrophoretic transport. First, electrophoretic transport requires that the reactants be charged in order to be affected by the electric fields; however, conventional reactants of interest for combinatorial chemistry are usually uncharged molecules not useable in an electrophoretic system. Second, the Heller method does not, and cannot, address the large amount of chemical crosstalk that inherently occurs because of the spatial distribution of the electric fields involved in the electrophoretic transport of the reagents for binding. In a system utilizing electrophoresis, one cannot use protecting groups to protect the reactive functional groups at the microlocations since there is no mechanism for removing the protective groups; yet, the use of electrophoresis results in various binding entities and/or reactants being located throughout the solution used as they migrate, often coming into contact with unselected reaction sites. Thus, the combination of the lack of protecting groups and the spatial distribution of the electric fields inherent to electrophoresis allow such binding reactions to occur randomly, compromising the fidelity of any polymer being synthesized.

From the above, it is seen that there is an existing need and desire for an improved method for synthesizing a variety of chemical sequences at known locations that uses highly efficient deprotection and coupling mechanisms. It is further seen that there is an existing need and desire for a method for synthesizing a variety of chemical sequences at known locations that is cost-effective and practical, and that allows use of a smaller sized apparatus affording more efficient production in a specific area and time, while maintaining the fidelity of the chemical sequences produced. As should be clear to those skilled in the art, the above discussion directed to polypeptide synthesis from monomers is equally applicable to oligonucleotide, and more specifically, deoxyribonucleic acid (DNA) synthesis from deoxyribonucleotide monomers.

It is therefore an object of the present invention to provide an improved method for the placement of a material at a specific location on a substrate. It is further an object of the present invention to provide an improved method for the rapid synthesis of an array of separate, diverse and pure polymers or oligonucleotides on a substrate.

It is still a further object of the invention to provide a substrate for separate and pure polymer or oligonucleotide or DNA synthesis that contains a multi-electrode array that allows electrodes to be placed in very close proximity for use in combinatorial chemistry. It is still another object of the invention to provide a substrate for separate and pure polymer or DNA synthesis that contains a multi-electrode array of electrodes in very close proximity, that allows for automation of a polymer or DNA synthesis process, and that can be used in functional genomics, diagnostics, gene screening, drug discovery and screening for materials useful for research, industrial, commercial and therapeutic uses.

Additional features and advantages of the present invention will be set forth in part in the description that follows, and in part will be apparent from the description, or may be learned by practice of the invention. The objectives and other advantages of the present invention will be realized and attained by means of the elements and combinations particularly pointed out in the written description and appended claims.

SUMMARY OF THE INVENTION

The foregoing objects have been accomplished in accordance with this invention by providing a method for elec-

trochemical placement of a material at a specific location on a substrate, which comprises the steps of:

providing a substrate having at its surface at least one electrode that is proximate to at least one molecule that is reactive with an electrochemically generated reagent,

applying a potential to the electrode sufficient to generate electrochemical reagents capable of reacting to the at least one molecule proximate to the electrode, and

producing a chemical reaction thereby.

The present invention also includes a method for the electrochemical placement of a material at a specific location on a substrate comprising the steps of:

providing a substrate having at its surface at least one electrode that is proximate to at least one molecule bearing at least one protected chemical functional group,

applying a potential to the electrode sufficient to generate electrochemical reagents capable of deprotecting at least one of the protected chemical functional groups of the molecule, and

bonding the deprotected chemical functional group with a monomer or a pre-formed molecule.

The present invention also includes a method for electrochemical synthesis of an array of separately formed polymers on a substrate, which comprises the steps of:

placing a buffering or scavenging solution in contact with an array of electrodes that is proximate to a substrate surface, said surface being proximate to one or more molecules bearing at least one protected chemical functional group attached thereto,

selectively deprotecting at least one protected chemical functional group on at least one of the molecules;

bonding a first monomer having at least one protected chemical functional group to one or more deprotected chemical functional groups of the molecule;

selectively deprotecting a chemical functional group on the bonded molecule or another of the molecules bearing at least one protected chemical functional group;

bonding a second monomer having at least one protected chemical functional group to a deprotected chemical functional group of the bonded molecule or the other deprotected molecule; and

repeating the selective deprotection of a chemical functional group on a bonded protected monomer or a bonded protected molecule and the subsequent bonding of an additional monomer to the deprotected chemical functional group until at least two separate polymers of desired length are formed on the substrate surface.

Another embodiment of the present invention also includes a method for electrochemical synthesis of an array of separately formed oligonucleotides on a substrate, which comprises the steps of:

placing a buffering or scavenging solution in contact with an array of electrodes that is proximate to a substrate surface, said surface being proximate to one or more molecules bearing at least one protected chemical functional group attached thereto,

selectively deprotecting at least one protected chemical functional group on at least one of the molecules;

bonding a first nucleotide having at least one protected chemical functional group to one or more deprotected chemical functional groups of the molecule;

selectively deprotecting a chemical functional group on the bonded molecule or another of the molecules bearing at least one protected chemical functional group;

bonding a second nucleotide having at least one protected chemical functional group to a deprotected chemical functional group of the bonded molecule or the other deprotected molecule; and

repeating the selective deprotection of a chemical functional group on a bonded protected nucleotide or a bonded protected molecule and the subsequent bonding of an additional nucleotide to the deprotected chemical functional group until at least two separate oligonucleotides of desired length are formed on the substrate surface.

A further embodiment of the present invention includes placing a "getter" structure such as a second electrode proximate to the array of electrodes or proximate to each of the electrodes individually. Such a "getter" structure may reduce chemical crosstalk between adjacent electrodes and/or prolong the life of semiconductor circuitry. Various semiconductor circuitry may be placed in a manner to control electrodes individually or corporately according to any one of the methods that are well known in the art. A "getter" structure in accordance with the present invention may be placed in an appropriate location either exposed to the external environment or internal to a semiconducting device.

By using the electrochemical techniques discussed herein, it is possible to place monomers, both those that can be used for polymer synthesis and those that can be decorated, and pre-formed molecules at small and precisely known locations on a substrate. It is therefore possible to synthesize polymers of a known chemical sequence at selected locations on a substrate. For example, in accordance with the presently disclosed invention, one can place nucleotides at selected locations on a substrate to synthesize desired sequences of nucleotides in the form of, for example, oligonucleotides.

It is to be understood that both the foregoing general description and the following detailed description are exemplary and explanatory only and are intended to provide further explanation of the present invention, as claimed.

BRIEF DESCRIPTION OF THE DRAWINGS

FIGS. 1*a* and 1*b* illustrate selective deprotection by electrochemically generated reagents (protons) generated at electrodes 1 and 4 to expose reactive functionalities (NH_2) on linker molecules (L) proximate electrodes 1 and 4. The substrate is shown in cross section and contains 5 electrodes.

FIGS. 2*a* and 2*b* illustrate the bonding of monomers (A) bearing protected chemical functional groups (P) with the deprotected linker molecules (bearing reactive functionalities) proximate electrodes 1 and 4.

FIGS. 3*a* and 3*b* illustrate selective deprotection by protons generated at electrodes 2 and 4 of a second set of reactive functionalities on the molecule and monomer proximate electrodes 2 and 4, respectively.

FIGS. 4*a* and 4*b* illustrate the bonding of monomers (B) bearing protected chemical functional groups (P) with the deprotected molecule and monomer proximate electrodes 2 and 4, respectively.

FIG. 5 illustrates a 5 electrode substrate bearing all possible combinations of monomers (A) and (B). The linker molecule proximate electrode 1 has a protected dimer, e.g., a dipeptide, containing two (A) monomers bonded thereto. The linker molecule proximate electrode 2 has a protected dimer containing a (B) monomer bonded to the linker molecule (L) and a protected (A) monomer bonded to said (B) monomer. The linker molecule proximate electrode 3,

which represents a control electrode, demonstrates a linker molecule where no synthesis occurs because no potential is applied to the proximate electrode. The linker molecule proximate electrode 4 has a protected dimer containing an (A) monomer bonded to a linker molecule (L) and a protected (B) monomer bonded to said (A) monomer. The linker molecule proximate electrode 5 has a protected dimer containing two (B) monomers bonded to a linker molecule (L).

FIG. 6 illustrates a top view diagram of a substrate having at its surface a 10×10 electrode array, having 100 electrodes. A side view of an exemplary electrode at the surface of the substrate is also shown.

FIG. 7 illustrates a substrate having a permeable attachment layer or membrane having CBZ-protected leucine monomers (L) bonded thereto. The layer/membrane overlays the electrodes at the surface of the substrate.

FIG. 8 illustrates a substrate having a permeable attachment layer or membrane overlaying the electrodes at the surface, which layer/membrane contains leucine monomers (L) bearing reactive amine functionalities, e.g., following removal of protecting groups (P═CBZ) at monomers proximate electrodes 2, 3, 5, 6, and 7 and counter electrodes 1 and 10.

FIG. 9 illustrates modification of monomers proximate electrodes 2,3, 5, 6, and 7 following CBZ-protected phenylalanine monomers (F) have bonded with the reactive amine functionalities on the leucine monomers proximate these electrodes (a dipeptide is formed).

FIG. 10 illustrates modification of the substrate surface by CBZ-protected tripeptides, glycine-phenylalanine-leucine (G-F-L) proximate electrodes 3, 5, 6, and 7.

FIG. 11 illustrates modification of the substrate surface by CBZ-protected pentapeptides, tyrosine-glycine-glycine-phenylalanine-leucine (Y-G-G-F-L) proximate electrodes 6 and 7.

FIG. 12 illustrates a protected leu-enkephalin epitope proximate electrode 7 and counter electrodes 1 and 10, and a deprotected leu-enkephalin epitope proximate electrode 6.

FIG. 13 illustrates representative results as would be observed using an epifluorescent microscope following exposure to the antibody and fluorescent conjugate in accordance with Example 1.

FIG. 14 is a digitally captured white light photomicrograph of an uncoated electrode array chip showing approximately seventy electrodes. This photomicrograph was taken using a 4× objective by an Olympus BX60 microscope with a Pulnix TM-745 integrating CCD camera. Note, there is electrical circuitry associated with these independently addressable electrodes.

FIG. 15 is a digitally captured epifluorescent photomicrograph of the same array of electrodes pictured in FIG. 14, at the same magnification. This photomicrograph shows that on an uncoated electrode array chip, without any fluorescent coating material thereon, the electrodes are dark. The darkness of the electrodes is explained by the metal of the electrode (platinum) quenching any fluorescence present.

FIG. 16 is a digitally captured epifluorescent photomicrograph of electrodes in the same array as in FIGS. 14 and 15, but taken using a 10× objective and showing only sixteen electrodes. This photomicrograph is of a chip that is coated with a fluorescent membrane material, i.e., there are fluorescent labeled molecules attached to a membrane overlaying the electrodes. This photomicrograph shows that when the electrodes are coated with a membrane containing fluorescent material, the area proximate/over the electrodes is bright. The fluorescent material used for this photomicrograph was streptavidin molecules labeled with Texas Red dye.

FIG. 17 is a digitally captured white light photomicrograph similar to FIG. 14, except that these electrodes are hard wired, as shown by the leads connecting the electrodes to the electrical source located off the micrograph. In addition, this photomicrograph was taken using a 10× objective. These hardwired electrodes are located on the side of the electrode array chips. Note, there is no circuitry associated with these hard wired-electrodes.

FIGS. 18*a* and 18*b* depict the chip/pin grid array (PGA) package assembly. As is shown in FIG. 18*a*, the chip is attached to the PGA package with glue on the opposite side of the chip from the active area (active area is the area having electrodes at its surface), which leaves the active electrode area protruding from the end of the PGA package in a manner that allows the active area of the chip to be dipped or immersed into solutions. The electrical wires that connect the bond pads on the chip to the bond pads on the PGA package are encased in epoxy. The pins shown in FIG. 18*b* are located on the opposite side of the PGA package shown in FIG. 18*a*.

FIGS. 19*a* and 19*b* represent digitally captured epifluorescent photomicrographs showing an electrode array chip before (FIG. 19*a*) and after (FIG. 19*b*) application of voltage and performance of a deprotection step. Prior to application of any voltage, a 0.05M aqueous sodium phosphate buffer at a pH of 8.0 was placed in contact with all the electrodes of the array to enable production of electrochemical reagents. FIG. 19*b* shows the electrode array after all of the electrodes in the array were exposed to the same voltage and deprotection occurred at each electrode in the array. A voltage of 2.8 volts was applied for 10 minutes. This photomicrograph was taken using a 4× objective and using a 1 second integration time.

FIG. 20 represents a digitally captured epifluorescent photomicrograph showing a hardwired electrode array chip wherein the anodes (the dark electrodes) and the cathodes were alternating electrodes. The depicted checkerboard pattern was obtained following application of 2.8 volts for 10 minutes. The objective used to obtain this photomicrograph was 4× and the integration time was 1 second. Note, the localization of the acid at the anodes. The precision of the localization achieved in accordance with the present invention allowed the checkerboard pattern to be obtained.

FIG. 21 represents a digitally captured epifluorescent photomicrograph showing the same hardwired electrode array chip as in FIG. 20, but this photomicrograph was taken using a 10× objective with a 700 millisecond integration time.

FIG. 22 is a digitally captured epifluorescent photomicrograph of an uncoated electrode array chip showing an array of hardwired electrodes. (The neighboring electrode array is also shown in this figure.) The orientation of the array shown allows accurate reading of the brightness of the electrodes. The electrodes shown are dark. The three electrodes to which electrical connection was provided, and of which brightness or darkness observations were made, are labeled "T1", "T2", and "T4".

FIG. 23 is a digitally captured epifluorescent photomicrograph of a chip that is coated with a fluorescent membrane containing Texas Red labeled streptavidin molecules that are attached to the electrodes via trityl linker molecules. Electrodes T2 and T4 have a strong bright signal. Electrode T1 is dark. No voltage has been applied to the electrodes yet.

FIG. 24 is a digitally captured epifluorescent photomicrograph of the chip shown in FIG. 23 after positive voltage has been applied to electrodes T2 and T4. Positive voltage produced protons at these electrodes. Electrodes T2 and T4 are dark because the trityl linker molecule has dissociated from the membrane overlaying the electrodes. Electrode T1 was used as the counter electrode. Note that the dark areas are confined to electrodes T2 and T4, i.e., there is very little chemical cross talk occurring between neighboring electrodes.

FIGS. 25*a* and 25*b* represent digitally captured epifluorescent photomicrographs showing hardwired electrodes before (FIG. 25*a*) and after (FIG. 25*b*) a deprotection step performed in accordance with the reaction conditions, i.e., electrolyte, of the prior art, Southern WO 93/22480. These photomicrographs, taken through a 10× objective, show the imprecision and randomness caused by "chemical crosstalk" between the electrodes. The large areas of black-out and white-out surrounding the electrodes in these photomicrographs represent the excursion of the electrochemical reagents (protons) away from the electrode at which they were generated.

FIGS. 26*a* and 26*b* represent digitally captured epifluorescent photomicrographs taken through a 20× objective with a 100 millisecond integration time of the same hardwired electrodes as shown in FIGS. 25*a* and 25*b*.

FIG. 27 represents electrochemical reduction of vitamin B_{12} at the cathodes of electrode array chips described in Experiment 5. The chips were exposed to Texas Red labeled streptavidin and this photomicrograph was obtained. Bright spots at the cathodes indicate the presence of biotin bound to the overlaying membrane.

FIG. 28 confirms that the vitamin B_{12} catalyst was necessary for the results depicted in FIG. 27. No vitamin B_{12} was added to the solution and a voltage difference of 3.0 volts between the anode and the cathode was set. No observable current passed between the electrodes. At this potential difference, there are no electroactive species in solution without vitamin B_{12}. The chip was then exposed to Texas Red labeled streptavidin and washed. No evidence of carbon—carbon bond formation is seen.

FIG. 29 confirms that the biotin anhydride substrate was necessary to obtain the results depicted in FIG. 27. A chip was immersed in a DMF solution identical to the DMF solution used to form carbon—carbon bonds, except that there was no biotin anhydride added to the solution. A voltage difference of 3.0 volts between the anode and the cathode was set. The chip was then exposed to Texas Red labeled streptavidin and washed. No evidence of carbon—carbon bond formation is seen.

FIG. 30 confirms that the activated olefin was necessary to obtain the results depicted in FIG. 27. A chip was immersed in a DMF solution identical to the DMF solution used to form carbon—carbon bonds, except that there was no activated olefin attached to the overlaying membrane. A voltage difference of 3.0 volts between the anode and the cathode was set. The chip was then exposed to Texas Red labeled streptavidin and washed. No evidence of carbon—carbon bond formation is seen.

FIG. 31 depicts a transistor design representative of those used in electrode arrays in accordance with the present invention. A key feature is the placement of a "getter" structure which in this case is an electrode proximate to and in a ring around the selected electrode.

FIG. 32 demonstrates ionic contamination at the monitoring transistor when the chips were dipped into aqueous salt solutions. This illustrates the shift in the threshold voltage of the transistor monitoring device after a 20 minute exposure to a 0.1 M $NaPO_4$ solution. The measured data is fit to a subthreshold MOS curve of the form $V=V_0\ln(I/I_0)$.

FIG. 33 demonstrates that the getter current continues to rise for several hours after initial exposure to ions indicating a concomitant lowering in the threshold voltage of the gate. These results indicate that the sodium ions are diffusing away from the gate region. The time course of the current rise follows an approximately logarithmic course for the first few hours.

FIG. 34*a* depicts a top view of an electrode array having a "getter" structure 2 which forms a substantial sheet around the individual electrodes 1 configured in the electrode array. Such a "getter" structure 2 in a sheet functions to capture ions which may diffuse into or toward the semiconductor circuitry.

FIG. 34*b* depicts a cross-section of a selected electrode having a "getter" structure 2 placed substantially at the interface between an insulating dielectric layer 3 and the metal surface of the semiconductor. The "getter" structure in this figure forms a substantially solid sheet with holes allowing the selected electrodes to contact the environment. This and similar structures extend the lifetime of semiconductor circuitry thereby making practical the submersion of a chip in ionic solutions.

FIGS. 35*a* and 35*b* represent an exemplary selected electrode 1 useful according to the present invention having a "getter" structure 2 substantially exposed to the external environment. Such a configuration may be especially useful to control electrochemically generated reagents diffusing between electrodes in an array as well as ions diffusing toward and into semiconductor circuitry. FIG. 35*a* represents a top view showing a selected electrode having a "getter" structure 2 forming a substantial ring around the selected electrode. FIG. 35*b* represents a cross section of the same showing a selected electrode having a "getter" structure 2 forming a substantial ring around the selected electrode 1.

FIGS. 36*a* and 36*b* represent an exemplary selected electrode 1 useful according to the present invention having a "getter" structure 2 beneath the surface of the electrode. Such a configuration may be especially useful to control ion diffusion toward and into semiconductor circuitry. FIG. 36*a* represents a top view showing a selected electrode having a "getter" structure 2 forming a substantial ring around the selected electrode 1 and beneath the top surface of the selected electrode. FIG. 35*b* represents a cross section of the same showing a selected electrode having a "getter" structure 2 forming a substantial ring around the selected electrode 1. The "getter" structure is placed beneath the external surface of the insulating dielectric 3 in this configuration.

FIG. 37 illustrates how the methods of the present invention function to isolate contaminating ions and thereby prevent chemical cross-talk between neighboring selected electrodes. The buffering and/or scavenging solutions of the present invention alone or combination with a "getter" structure effectively isolate reactive electrochemically generated reagents thereby allowing multiple chemical reactions in close proximity.

DETAILED DESCRIPTION OF THE INVENTION

The present invention provides methods for the preparation and use of a substrate having one or a plurality of chemical species in selected regions. The present invention

is described herein primarily with regard to the preparation of molecules containing sequences of amino acids, but could be readily applied to the preparation of other polymers, as well as to the preparation of sequences of nucleic acids. Such polymers include, for example, both linear and cyclic polymers of nucleic acids, polysaccharides, phospholipids, and peptides having either alpha-, beta-, or omega- amino acids, polyurethanes, polyesters, polycarbonates, polyureas, polyamides, polyethyleneimines, polyarylene sulfides, polysiloxanes, polyimides, polyacetates, or other polymers which will be apparent upon review of this disclosure. In a preferred embodiment, the invention herein is used in the synthesis of peptides. In another preferred embodiment, the present invention is used for the synthesis of oligonucleotides and/or DNA.

The present invention is directed to placing molecules, selected generally from monomers, linker molecules and pre-formed molecules, including, in particular, nucleic acids, at a specific location on a substrate. The present invention is more particularly directed to the synthesis of polymers at a specific location on a substrate, and in particular polypeptides, by means of a solid phase polymerization technique, which generally involves the electrochemical removal of a protecting group from a molecule provided on a substrate that is proximate at least one electrode. The present invention is also particularly directed to the synthesis of oligonucleotides and/or DNA at selected locations on a substrate, by means of the disclosed solid phase polymerization technique.

Electrochemical reagents capable of electrochemically removing protecting groups from chemical functional groups on the molecule are generated at selected electrodes by applying a sufficient electrical potential to the selected electrodes. Removal of a protecting group, or "deprotection," in accordance with the invention, occurs at selected molecules when a chemical reagent generated by the electrode acts to deprotect or remove, for example, an acid or base labile protecting group from the selected molecules.

In one embodiment of the present invention, a terminal end of a monomer nucleotide, or linker molecule (i.e., a molecule which "links," for example, a monomer or nucleotide to a substrate) is provided with at least one reactive functional group, which is protected with a protecting group removable by an electrochemically generated reagent. The protecting group(s) is exposed to reagents electrochemically generated at the electrode and removed from the monomer, nucleotide or linker molecule in a first selected region to expose a reactive functional group. The substrate is then contacted with a first monomer or pre-formed molecule, which bonds with the exposed functional group(s). This first monomer or pre-formed molecule may also bear at least one protected chemical functional group removable by an electrochemically generated reagent.

The monomers or pre-formed molecules can then be deprotected in the same manner to yield a second set of reactive chemical functional groups. A second monomer or pre-formed molecule, which may also bear at least one protecting group removable by an electrochemically generated reagent, is subsequently brought into contact with the substrate to bond with the second set of exposed functional groups. Any unreacted functional groups can optionally be capped at any point during the synthesis process. The deprotection and bonding steps can be repeated sequentially at this site on the substrate until polymers or oligonucleotides of a desired sequence and length are obtained.

In another embodiment of the present invention, the substrate having one or more molecules bearing at least one protected chemical functional group bonded thereto is proximate an array of electrodes, which array is in contact with a buffering or scavenging solution. Following application of an electric potential to selected electrodes in the array sufficient to generate electrochemical reagents capable of deprotecting the protected chemical functional groups, molecules proximate the selected electrodes are deprotected to expose reactive functional groups, thereby preparing them for bonding. A monomer solution or a solution of pre-formed molecules, such as proteins, nucleic acids, polysaccharides, and porphyrins, is then contacted with the substrate surface and the monomers or pre-formed molecules bond with the deprotected chemical functional groups.

Another sufficient potential is subsequently applied to select electrodes in the array to deprotect at least one chemical functional group on the bonded molecule or another of the molecules bearing at least one protected chemical functional group. A second monomer or pre-formed molecule having at least one protected chemical functional group is subsequently bonded to a deprotected chemical functional group of the bonded molecule or the other deprotected molecule. The selective deprotection and bonding steps can be repeated sequentially until polymers or oligonucleotides of a desired sequence and length are obtained. The selective deprotection step is repeated by applying another potential sufficient to effect deprotection of a chemical functional group on a bonded protected monomer or a bonded protected molecule. The subsequent bonding of an additional monomer or pre-formed molecule to the deprotected chemical functional group(s) until at least two separate polymers or oligonucleotides of desired length are formed on the substrate. FIGS. 1--5 generically illustrate the above-discussed embodiments.

Preferred embodiments of the present invention use a buffering or scavenging solution in contact with each electrode, which is buffered towards the electrochemically generated reagents, in particular, towards protons and/or hydroxyl ions, and that actively prevents chemical cross-talk caused by diffusion of the electrochemically generated ions from one electrode to another electrode in an array. For example, when an electrode exposed to an aqueous or partially aqueous media is biased to a sufficiently positive (or negative) potential, protons (or hydroxyl ions) are produced as products of water hydrolysis. Protons, for example, are useful for removing electrochemical protecting groups from several molecules useful in combinatorial synthesis, for example, peptides, nucleic acids, and polysaccharides.

In order to produce separate and pure polymers, it is desirable to keep these protons (or hydroxyl ions) confined to the area immediately proximate the selected electrode(s) in order to minimize, and, if possible to eliminate, chemical cross-talk between nearby electrodes in an array. The spatial extent of excursion of electrochemically generated reagents can be actively controlled by the use of a buffering or scavenging solution that reacts with the reagents that move away from the selected electrodes, thus preventing these reagents from reacting at a nearby electrode.

Another technique for confining these electrochemically generated reagents to the area immediately proximate the selected electrode(s) is to place a "getter" structure in proximity to the selected electrode(s) and substantially exposed to the external environment. Such a "getter" structure may be used in conjunction with or in place of a scavenging solution. A "getter" structure may be designed of any suitable material and formed into any suitable shape or size as skilled artisans will readily appreciate. The most important criteria for such a "getter" structure is that it

function to scavenge electrochemically generated reagents that may diffuse away from the selected electrode(s). The "getter" structure may function passively by reacting chemically with the electrochemically generated reagents. Alternatively, the "getter" structure may function actively to scavenge the electrochemically generated reagents. This may be performed by applying sufficient potential to the "getter" structure to cause electrochemical scavenging. Another function of the "getter" structure may be to prevent the diffusion of ions toward or into circuitry such as transistors that may be operably linked to the selected electrode (s). In accordance with this function, the "getter" structure may be placed substantially at the interface between an insulating dielectric and a metallization layer operably linked to the selected electrode(s).

Some preferred embodiments of such a "getter" structure include a metal sheet that may cover or substantially cover the surface of the circuitry of the electrode device used in the present invention. An example of such a structure is depicted in FIG. 34. This metal sheet may have holes existing where the electrodes are placed. The electrodes may in turn be separated from the "getter" structure by a dielectric as is demonstrated in FIG. 31. Even more preferred embodiments of such a "getter" structure include a ring electrode around the selected electrode(s). A ringed "getter" structure offers at least two advantages over other embodiments such as those described above. First, much of the diffusion of ions occurs along defect sites that form at the interface between the selected electrode(s) and the dielectric. Second, it is relatively easy to monitor the effects of the "getter" structure on the environment when such a structure is utilized. Such a structure is exemplified in FIGS. 31 and 34–36.

The "getter" structure according to the present invention further solves the problem associated with exposing semiconductor devices that may be used in conjunction with the selected electrodes to environments that contain ions that diffuse into the device. In particular, ions from solutions to which a semiconductor device is exposed may diffuse into regions of a semiconductor device that have been doped with ions in a precise manner to impart particular electrical properties to these regions. An important example is the gate of a metal oxide semiconductor (MOS) transistor circuit element. Here either positive or negative ions (e.g., p-doped or n-doped) have been diffused into the gate region to make the region semiconducting. The threshold voltage and current-voltage characteristics of the transistor gate depend in a sensitive way on doping levels. The long term reliability of many semiconductor devices depends on isolating them effectively from ionic contamination. For example, the adhesives and encapsulants used in the semiconductor industry are treated to render the ion concentrations in these materials as low as possible, often less than parts per million.

Semiconductor transistors are rapidly destroyed when silicon chips are used in ion-containing solutions. Semiconductor transistors are presently manufactured with a thin layer of partially conductive material in their transistor junctions. This partially conductive layer is infused or doped with a concentration of particles, i.e. ions, to achieve a balanced level of conductivity. This is typically done at present by doping the junction with a substance rich in ions such as arsenic, boron or phosphorous. When too many ions are present, the material functions as a metal and becomes highly conductive. When there are too few ions present, the material functions as an insulator and demonstrates very low conductivity. In order to perform properly as a transistor, the material must achieve a very specific level of conductivity intermediate between that of a metal and that of an insulator. Contamination of the partially conductive layer at the transistor junction by ions diffusing into a semiconductor device changes conductivity of the junction and thus destroys the transistor.

Ion contamination represents a serious obstacle for exposing semiconductor circuitry to hostile or ion-containing solutions. This presents a major impediment to devices according to some embodiments of the present invention wherein semiconductor circuitry may be operably linked to selected electrode(s), which are in turn immersed in high concentration ionic solutions for extended periods of time. As a result, embodiments according to the present invention that utilize structures such as a "getter" structure are designed both to monitor and to obviate ion contamination are particularly preferred.

Such devices may be designed to work by scavenging ions that diffuse into the device from solutions to which the electrode and associated circuitry may be exposed. Contaminating ions may be scavenged passively by reacting chemically with a material that is placed between them and the active circuitry. Alternatively, they can be scavenged actively by applying a voltage to a second electrode placed proximate to the selected electrode(s) that sets up an electric field that causes ions to migrate to the electrode and away from the active circuitry. Ion contamination can be monitored by placing transistor gates adjacent to the getter structure and monitoring shifts in threshold voltage. Such "getter" structures may be designed by skilled artisans of any suitable material in any suitable size or shape and thereby be adapted to any electrode geometries. Moreover, it is generally preferable to place the "getter" structure beneath an electrically insulating or dielectric layer such as a silicon nitride that generally covers a semiconductor and thereby separates the semiconductor from the environment, and, in particular, from the ionic solutions required in the practice of the present invention. It is particularly preferable to place the "getter" structure in a ring either substantially beneath or substantially within a dielectric layer and substantially surrounding the select electrode(s). An exemplary cross-section of such a structure is presented in FIG. 34. As a result of the "getter" structure used in accordance with the present invention, selected electrode(s) may be advantageously controlled by automated computer circuitry while maintaining a viable lifespan for the same in the environment of ionic solutions. Thus, it is practical to synthesize a variety of chemical molecules on the surface of electrodes integrated into or operably linked to a computer chip in accordance with methods well known to those of skill in the art.

The present invention advantageously minimizes, and preferably eliminates, chemical cross-talk between nearby areas of polymer or nucleic acid sequence synthesis on a substrate, thus enabling the synthesis of separate arrays of pure polymers or nucleic acid sequences in a small specified area on a substrate using conventional electrochemically generated reagents and known electrochemical reactions. The ability of the inventive methods to place materials at specific locations on a substrate enables the inventive method to be used in several areas of synthesis in addition to polymer synthesis. Several examples of this synthesis include DNA and oligonucleotide synthesis, monomer decoration, which involves the addition of chemical moieties to a single monomer, and inorganic synthesis, which involves the addition of, for example, metals to porphyrins.

Other embodiments of the present invention contemplate an array of electrodes of small micron size, for example, ranging from 1 to 100 microns in diameter, and separated by

many microns. However, it is also contemplated that electrodes separated by only submicron distances can be used, if desired. This arrangement affords a large quantity of separate and pure polymers or nucleic acid sequences to be synthesized simultaneously in a small area on a substrate in accordance with the inventive method. This capability renders the inventive method easily automated. The ability of the present invention to be automated easily while retaining the capability of producing separate and diverse arrays of pure polymers and nucleic acid sequences makes the present invention ideal for use in the rapidly developing areas of combinatorial chemistry and functional genomics.

Essentially, any conceivable substrate may be employed in accordance with the present invention. The substrate may be biological, nonbiological, organic, inorganic, or a combination of any of these, existing as particles, strands, precipitates, gels, sheets, tubing, spheres, containers, capillaries, pads, slices, films, plates, slides, etc. The substrate may have any convenient shape, such as a disc, square, sphere, circle, etc. The substrate is preferably flat, but may take on a variety of alternative structure configurations. For example, the substrate may contain raised or depressed regions on which synthesis may take place. The substrate and its surface preferably form a rigid support on which to carry out the reactions described herein. The substrate and the area for synthesis of each individual polymer or small molecule may be of any size and shape. Moreover, a substrate may comprise different materials at different regions.

Contemplated materials, which are preferably used as substrates and which are capable of holding and insulating electrically the electrodes, include: undoped semiconductors, such as silicon nitride, silicon oxide, silicon, diamond, chalcopyrites, wurtzites, sphalerites, halites, Group III-V compounds, and Group I-VI compounds; glass, such as, cobalt glass, pyrex glass, vycor glass, borosilicate glass and quartz; ceramics, such as, alumina, porcelain, zircon, corderite, titanates, metal oxides, clays, and zeolites; polymers, such as, paralyene, high density polyethylene, teflons, nylons, polycarbonates, polystyrenes, polyacylates, polycyanoacrylates, polyvinyl alcohols, polyimides, polyamides, polysiloxanes, polysilicones, polynitriles, polyvinyl chlorides, alkyd polymers, celluloses, expoxy polymers, melamines, urethanes, copolymers and mixtures of any of the above with other polymers, and mixtures of any of the above with glass or ceramics; and waxes, such as, apeizon. Other substrate materials will be readily apparent to those of skill in the art upon review of this disclosure.

The substrate of the invention is proximate to at least one electrode, i.e., an electrically conducting region of the substrate that is substantially surrounded by an electrically insulating region. The electrode(s), by being "proximate" to the substrate, can be located at the substrate, i.e., embedded in or on the substrate, can be next to, below, or above the substrate, but need to be in close enough proximity to the substrate so that the reagents electrochemically generated at the electrode(s) can accomplish the desired deprotection of the chemical functional groups on the monomer(s) and/or molecule(s).

In addition to being proximate to at least one electrode, the substrate has on a surface thereof, at least one molecule, and preferably several molecules, bearing at least one chemical functional group protected by an electrochemically removable protecting group. These molecules bearing protected chemical functional groups also need to be proximate to the electrode(s). In this regard, the molecules on the surface of the substrate need to be in close enough proximity to the electrode(s) so that the electrochemical reagents generated at the electrode can remove the protecting group from at least one protected functional group on the proximate molecule(s).

The molecules bearing a protected chemical functional group that are attached to the surface of the substrate may be selected generally from monomers, linker molecules and pre-formed molecules. Preferably, the molecules attached to the surface of the substrate include monomers, nucleotides, and linker molecules. All of these molecules generally bond to the substrate by covalent bonds or ionic interactions. Alternatively, all of these molecules can be bonded, also by covalent bonds or ionic interactions, to a layer overlaying the substrate, for example, a permeable membrane layer, which layer can be adhered to the substrate surface in several different ways, including covalent bonding, ionic interactions, dispersive interactions and hydrophilic or hydrophobic interactions. In still another manner of attachment, a monomer or pre-formed molecule may be bonded to a linker molecule that is bonded to either the substrate or a layer overlaying the substrate.

The monomers, linker molecules and pre-formed molecules used herein, are preferably provided with a chemical functional group that is protected by a protecting group removable by electrochemically generated reagents. If a chemical functional group capable of being deprotected by an electrochemically generated reagent is not present on the molecule on the substrate surface, bonding of subsequent monomers or pre-formed molecules cannot occur at this molecule. Preferably, the protecting group is on the distal or terminal end of the linker molecule, monomer, or pre-formed molecule, opposite the substrate. That is, the linker molecule preferably terminates in a chemical functional group, such as an amino or carboxy acid group, bearing an electrochemically removable protective group. Chemical functional groups that are found on the monomers, linker molecules and pre-formed molecules include any chemically reactive functionality. Usually, chemical functional groups are associated with corresponding protective groups and will be chosen or utilized based on the product being synthesized. The molecules of the invention bond to deprotected chemical functional groups by covalent bonds or ionic interactions.

Monomers used in accordance with the present invention to synthesize the various polymers contemplated include all members of the set of small molecules that can be joined together to form a polymer. This set includes, but is not limited to, the set of common L-amino acids, the set of D-amino acids, the set of synthetic amino acids, the set of nucleotides and the set of pentoses and hexoses. As used herein, monomers include any member of a basis set for synthesis of a polymer. For example, trimers of L-amino acids form a basis set of approximately 8000 monomers for synthesis of polypeptides. Different basis sets of monomers may be used at successive steps in the synthesis of a polymer using the inventive method. The number of monomers that can be used in accordance with the inventive synthesis methods can vary widely, for example from 2 to several thousand monomers can be used, but in more preferred embodiments, the number of monomers will range from approximately 4 to approximately 200, and, more preferably, the number of monomers will range from 4–20.

Additional monomers that can be used in accordance with the invention also include the set of monomers that can be decorated, i.e., monomers to which chemical moieties can be added, such as prostaglandins, benzodiazapines, thrombox-

anes and leukotrienes. Combinations of monomers useful for polymer synthesis and monomers that can be decorated are also contemplated by the invention. The above-discussed monomers may be obtained in unprotected form from most any chemical supply company, and most, if not all, can be obtained in protected form from Bachem, Inc., Torrance, Calif. Phosphoramidite monomers for nucleic acid synthesis can be obtained from Applied Biosystems, Inc., Foster City, Calif.

In a preferred embodiment of the invention, the monomers are amino acids containing a protective group at its amino or carboxy terminus that is removable by an electrochemically generated reagent. A polymer in which the monomers are alpha amino acids and are joined together through amide bonds is a peptide, also known as a polypeptide. In the context of the present invention, it should be appreciated that the amino acids may be the L-optical isomer or the D-optical isomer or a mixture of the two. Peptides are at least two amino acid monomers long, and often are more than 20 amino acid monomers long.

Furthermore, essentially any pre-formed molecule can be bonded to the substrate, a layer overlaying the substrate, a monomer or a linker molecule. Pre-formed molecules include, for example, proteins, including in particular, receptors, enzymes, ion channels, and antibodies, nucleic acids, polysaccharides, porphyrins, and the like. Pre-formed molecules are, in general, formed at a site other than on the substrate of the invention. In a preferred embodiment, a pre-formed molecule is bonded to a deprotected functional group on a molecule, monomer, or another pre- formed molecule. In this regard, a pre-formed molecule that is already attached to the substrate may additionally bear at least one protected chemical functional group to which a monomer or other pre-formed molecule may bond, following deprotection of the chemical functional group.

Protective groups are materials that bind to a monomer, a linker molecule or a pre-formed molecule to protect a reactive functionality on the monomer, linker molecule or pre-formed molecule, which may be removed upon selective exposure to an activator, such as an electrochemically generated reagent. Protective groups that may be used in accordance with the present invention preferably include all acid and base labile protecting groups. For example, peptide amine groups are preferably protected by t-butyloxycarbonyl (BOC) or benzyloxycarbonyl (CBZ), both of which are acid labile, or by 9-fluorenylmethoxycarbonyl (FMOC), which is base labile. Additionally, hydroxy groups on phosphoramidites may be protected by dimethoxytrityl (DMT), which is acid labile. Exocyclic amine groups on nucleosides, in particular on phosphoramidites, are preferably protected by dimethylformamidine on the adenosine and guanosine bases, and isobutyryl on the cytidine bases, both of which are base labile protecting groups. This protection strategy is known as fast oligonucleotide deprotection (FOD). Phosphoramidites protected in this manner are known as FOD phosphoramidites.

Additional protecting groups that may be used in accordance with the present invention include acid labile groups for protecting amino moieties: tertbutyloxycarbonyl,- tert-amyloxycarbonyl, adamantyloxycarbonyl, 1-methylcyclobutyloxycarbonyl, 2-(p-biphenyl)propyl(2) oxycarbonyl, 2- (p-phenylazophenylyl)propyl(2) oxycarbonyl, α,α-dimethyl-3,5-dimethyloxybenzyloxycarbonyl, 2-phenylpropyl(2)oxycarbonyl, 4-methyloxybenzyloxycarbonyl, benzyloxycarbonyl, furfuryloxycarbonyl, triphenylmethyl (trityl), p-toluenesulfenylaminocarbonyl, dimethylphosphinothioyl, diphenylphosphinothioyl, 2-benzoyl-1-methylvinyl, o-nitrophenylsulfenyl, and 1- naphthylidene; as base labile groups for protecting amino moieties: 9-fluorenylmethyloxycarbonyl, methylsulfonylethyloxycarbonyl, and 5- benzisoazolylmethyleneoxycarbonyl; as groups for protecting amino moieties that are labile when reduced: dithiasuccinoyl, p-toluene sulfonyl, and piperidino-oxycarbonyl; as groups for protecting amino moieties that are labile when oxidized: (ethylthio) carbonyl; as groups for protecting amino moieties that are labile to miscellaneous reagents, the appropriate agent is listed in parenthesis after the group: phthaloyl (hydrazine), trifluoroacetyl (piperidine), and chloroacetyl (2-aminothiophenol); acid labile groups for protecting carboxylic acids: tert-butyl ester; acid labile groups for protecting hydroxyl groups: dimethyltrityl; and basic labile groups for protecting phosphotriester groups: cyanoethyl.

As mentioned above, any unreacted deprotected chemical functional groups may be capped at any point during a synthesis reaction to avoid or to prevent further bonding at such molecule. Capping groups "cap" deprotected functional groups by, for example, binding with the unreacted amino functions to form amides. Capping agents suitable for use in the present invention include: acetic anhydride, n-acetylimidizole, isopropenyl formate, fluorescamine, 3-nitrophthalic anhydride and 3-sulfoproponic anhydride. Of these, acetic anhydride and n-acetylimidizole are preferred.

In accordance with the invention, the surface of the substrate is preferably provided with a layer of linker molecules. Linker molecules allow for indirect attachment of monomers or pre-formed molecules to the substrate or a layer overlaying the substrate. The linker molecules are preferably attached to an overlaying layer via silicon-carbon bonds, using, for example, controlled porosity glass (CPG) as the layer material. Linker molecules also facilitate target recognition of the synthesized polymers. Furthermore, the linker molecules are preferably chosen based upon their hydrophilic/ hydrophobic properties to improve presentation of synthesized polymers to certain receptors. For example, in the case of a hydrophilic receptor, hydrophilic linker molecules will be preferred so as to permit the receptor to approach more closely the synthesized polymer.

The linker molecules are preferably of sufficient length to permit polymers on a completed substrate to interact freely with binding entities exposed to the substrate. The linker molecules, when used, are preferably 650 atoms long to provide sufficient exposure of the functional groups to the binding entity. The linker molecules, which may be advantageously used in accordance with the invention include, for example, aryl acetylene, ethylene glycol oligomers containing from 2 to 20 monomer units, diamines, diacids, amino acids, and combinations thereof. Other linker molecules may be used in accordance with the different embodiments of the present invention and will be recognized by those skilled in the art in light of this disclosure.

According to another preferred embodiment, linker molecules may be provided with a cleavable group at an intermediate position, which group can be cleaved with an electrochemically generated reagent. This group is preferably cleaved with a reagent different from the reagent(s) used to remove the protective groups. This enables removal of the various synthesized polymers or nucleic acid sequences following completion of the synthesis by include: acetic anhydride, n-acetylimidizole, isopropenyl formate, fluorescamine, 3-nitrophthalic anhydride and 3-sulfoproponic anhydride. Of these, acetic anhydride and n-acetylimidizole are preferred.

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According to another preferred embodiment, linker molecules may be provided with a cleavable group at an intermediate position, which group can be cleaved with an electrochemically generated reagent. This group is preferably cleaved with a reagent different from the reagent(s) used to remove the protective groups. This enables removal of the various synthesized polymers or nucleic acid sequences following completion of the synthesis by way of electrochemically generated reagents. In particular, derivatives of the acid labile 4,4'-dimethyoxytrityl molecules with an exocyclic active ester can be used in accordance with the present invention. These linker molecules can be obtained from Perseptive Biosystems, Framingham, Mass. More preferably, N-succinimidyl-4[bis-(4-methoxyphenyl)-chloromethyl]-benzoate is used as a cleavable linker molecule during DNA synthesis. The synthesis and use of this molecule is described in *A Versatile Acid-Labile Linker for Modification of Synthetic Biomolecules*, by Brian D. Gildea, James M. Coull and Hubert Koester, *Tetrahedron Letters*, Volume 31, No. 49, pgs 7095–7098 (1990). Alternatively, other manners of cleaving can be used over the entire array at the same time, such as chemical reagents, light or heat.

The use of cleavable linker groups affords dissociation or separation of synthesized molecules, e.g., polymers or nucleic acid sequences, from the electrode array at any desired time. This dissociation allows transfer of the, for example, synthesized polymer or nucleic acid sequence, to another electrode array or to a second substrate. The second substrate could contain bacteria and serve to assay the effectiveness of molecules made on the original electrode array at killing bacteria. Alternatively, the second substrate could be used to purify the materials made on the original electrode array. Obviously, those skilled in the art can contemplate several uses for transferring the molecules synthesized on the original electrode to a second substrate.

The molecules of the invention, i.e., the monomers, linker molecules and pre- formed molecules, can be attached directly to the substrate or can be attached to a layer or membrane of separating material that overlays the substrate. Materials that can form a layer or membrane overlaying the substrate, such that molecules can be bound there for modification by electrochemically generated reagents, include: controlled porosity glass (CPG); generic polymers, such as, teflons, nylons, polycarbonates, polystyrenes, polyacylates, polycyanoacrylates, polyvinyl alcohols, polyamides, polyimides, polysiloxanes, polysilicones, polynitriles, polyelectrolytes, hydrogels, epoxy polymers, melamines, urethanes and copolymers and mixtures of these and other polymers; biologically derived polymers, such as, polysaccharides, polyhyaluric acids, celluloses, and chitons; ceramics, such as, alumina, metal oxides, clays, and zeolites; surfactants; thiols; self-assembled monolayers; porous carbon; and fullerine materials. The membrane can be coated onto the substrate by spin coating, dip coating or manual application, or any other art acceptable form of coating.

Reagents that can be generated electrochemically at the electrodes fall into two broad classes: oxidants and reductants. There are also miscellaneous reagents that are useful in accordance with the invention. Oxidants that can be generated electrochemically include iodine, iodate, periodic acid, hydrogen peroxide, hypochlorite, metavanadate, bromate, dichromate, cerium (IV), and permanganate. Reductants that can be generated electrochemically include chromium (II), ferrocyanide, thiols, thiosulfate, titanium (III), arsenic (III) and iron (II). The miscellaneous reagents include bromine, chloride, protons and hydroxyl ions. Among the foregoing reagents, protons, hydroxyl ions, iodine, bromine, chlorine and the thiols are preferred.

In accordance with preferred embodiments of the present invention, a buffering and/or scavenging solution is in contact with each electrode. The buffering and/or scavenging solutions that may be used in accordance with the invention are preferably buffered toward, or scavenge, ions such as protons and/or hydroxyl ions, although other electrochemically generated reagents capable of being buffered and/or scavenged are clearly contemplated. The buffering solution functions to prevent chemical cross- talk due to diffusion of electrochemically generated reagents from one electrode in an array to another electrode in the array, while a scavenging solution functions to seek out and neutralize/deactivate the electrochemically generated reagents by binding or reacting with them. Thus, the spatial extent of excursion of electrochemically generated reagents can be actively controlled by the use of a buffering solution and/or a scavenging solution. This function is graphically explained in FIG. 37. In accordance with the invention, the buffering and scavenging solutions may be used independently or together. Preferably, a buffering solution is used because the capacity of a buffering solution is more easily maintained, as compared with a scavenging solution.

Buffering solutions that can be used in accordance with the present invention include all electrolyte salts used in aqueous or partially aqueous preparations. Buffering solutions preferably used in accordance with the present invention include: acetate buffers, which typically buffer around pH 5; borate buffers, which typically buffer around pH 8; carbonate buffers, which typically buffer around pH 9; citrate buffers, which typically buffer around pH 6; glycine buffers, which typically buffer around pH 3; HEPES (4-[2-hydroxyethyl]-1pipirazine ethane sulfonic acid) buffers, which typically buffer around pH 7; MOPS (morpholinopropanesulfonic acid) buffers, which typically buffer around pH 7; phosphate buffers, which typically buffer around pH 7; TRIS (tris[hydroxymethyl]amino methane) buffers, which typically buffer around pH 8; and 0.1 M KI (potassium iodide) in solution, which buffers the

iodine concentration by the equilibrium reaction $I_2+I^-=I_3^-$, the equilibrium coefficient for this reaction being around 10^{-2}.

Alternatively, or in combination with a buffering solution, a scavenging solution may be used that contains species such as ternary amines that function as hydroxyl ion scavengers or sulfonic acids that function as proton scavengers in nonaqueous media. The rate at which a reagent/species is scavenged depends both on the intrinsic rate of the reaction occurring and on the concentration of the scavenger. For example, solvents make good scavengers because they are frequently present in high concentrations. Most molecules scavenge in a nonselective way, however, some molecules, such as superoxide dismutase and horseradish peroxidase, scavenge in a selective manner.

Of particular interest to the present invention are scavenger molecules that can scavenge the different reactive species commonly generated, for example, by water hydrolysis at electrodes, including hydroxyl radicals, superoxides, oxygen radicals, and hydrogen peroxide. Hydroxyl radicals are among the most reactive molecules known, their rate of reaction is diffusion controlled, that is, they react with the first reactant/species they encounter. When hydroxyl radicals are generated by water hydrolysis, the first molecule they usually encounter is a water molecule. For this reason, water is a rapid and effective scavenger of hydroxyl radicals. Superoxides are also a relatively reactive species, but can be stable in some nonequeous or partially aqueous solvents. In aqueous media, superoxides rapidly react with most molecules, including water. In many solvents, they can be scavenged selectively with superoxidase dismutase.

Oxygen radicals are a family of oxygen species that exist as free radicals. They can be scavenged by a wide variety of molecules such as water or ascorbic acid. Hydrogen peroxide is a relatively mild reactive species that is useful, in particular, in combinatorial synthesis. Hydrogen peroxide is scavenged by water and many types of oxidizing and reducing agents. The rate at which hydrogen peroxide is scavenged depends on the redox potential of the scavenger molecules being used. Hydrogen peroxide can also be scavenged selectively by horseradish peroxidase. Another electrochemically generated species that can be scavenged is iodine. Iodine is a mild oxidizing reagent that is also useful for combinatorial synthesis. Iodine can be scavenged by reaction with hydroxyl ions to form iodide ions and hypoiodite. The rate at which iodine is scavenged is pH dependent; higher pH solutions scavenge iodine faster. All of the scavenger molecules discussed above may be used in accordance with the present invention. Other scavenger molecules will be readily apparent to those skilled in the art upon review of this disclosure.

In accordance with the present invention, the buffering solutions are preferably used in a concentration of at least 0.01 mM. More preferably, the buffering solution is present in a concentration ranging from 1 to 100 mM, and still more preferably, the buffering solution is present in a concentration ranging from 10 to 100 mM. Most preferably, the buffering solution concentration is approximately 30 mM. A buffering solution concentration of approximately 0.1 molar, will allow protons or hydroxyl ions to move approximately 100 angstroms before buffering the pH to the bulk values. Lower buffering solution concentrations, such as 0.00001 molar, will allow ion excursion of approximately several microns, which still may be acceptable distance depending on the distance between electrodes in an array.

In accordance with the present invention, the concentration of scavenger molecules in a solution will depend on the specific scavenger molecules used since different scavenging molecules react at different rates. The more reactive the scavenger, the lower the concentration of scavenging solution needed, and vice versa. Those skilled in the art will be able to determine the appropriate concentration of scavenging solution depending upon the specific scavenger selected.

The at least one electrode proximate the substrate of the invention is preferably an array of electrodes. Arrays of electrodes of any dimension may be used, including arrays containing up to several million electrodes. Preferably, multiple electrodes in an array are simultaneously addressable and controllable by an electrical source. More preferably, each electrode is individually addressable and controllable by its own electrical source, thereby affording selective application of different potentials to select electrodes in the array. In this regard, the electrodes can be described as "switchable".

The arrays need not be in any specific shape, that is, the electrodes need not be in a square matrix shape. Contemplated electrode array geometries include: squares; rectangles; rectilinear and hexagonal grid arrays with any sort of polygon boundary; concentric circle grid geometries wherein the electrodes form concentric circles about a common center, and which may be bounded by an arbitrary polygon; and fractal grid array geometries having electrodes with the same or different diameters. Interlaced electrodes may also be used in accordance with the present invention. Preferably, however, the array of electrodes contains at least 100 electrodes in a 10x10 matrix. One embodiment of a substrate that may be used in accordance with the present invention having a 10x10 matrix of electrodes is shown in FIG. 6. A side view of an electrode at the surface of the substrate is also shown.

More preferably, the array of electrodes contains at least 400 electrodes in, for example, an at least 20x20 matrix. Even more preferably, the array contains at least 2048 electrodes in, for example, an at least 64x32 matrix, and still more preferably, the array contains at least 204,800 electrodes in, for example, an at least 640x320 array. Other sized arrays that may be used in accordance with the present invention will be readily apparent to those of skill in the art upon review of this disclosure.

Electrode arrays containing electrodes ranging in diameter from approximately less than 1 micron to approximately 100 microns (0.1 millimeters) are advantageously used in accordance with the present invention. Further, electrode arrays having a distance of approximately 10–1000 microns from center to center of the electrodes, regardless of the electrode diameter, are advantageously used in accordance with the present invention. More preferably, a distance of 50–100 microns exists between the centers of two neighboring electrodes.

As shown in the side view of FIG. 6, the electrodes may be flush with the surface of the substrate. However, in accordance with a preferred embodiment of the present invention, the electrodes are hemisphere shaped, rather than flat disks. More specifically, the profile of the hemisphere shaped electrodes is represented by an arctangent function that looks like a hemisphere. Those skilled in the art will be familiar with electrodes of this shape. Hemisphere shaped electrodes help assure that the electric potential is constant across the radial profile of the electrode. That is, hemisphere shaped electrodes help assure that the electric potential is not larger near the edge of the electrode than in the middle of the electrode, thus assuring that the generation of electrochemical reagents occurs at the same rate at all parts of the electrode.

Electrodes that may be used in accordance with the invention may be composed of, but are not limited to, noble metals such as iridium and/or platinum, and other metals, such as, palladium, gold, silver, copper, mercury, nickel, zinc, titanium, tungsten, aluminum, as well as alloys of various metals, and other conducting materials, such as, carbon, including glassy carbon, reticulated vitreous carbon, basal plane graphite, edge plane graphite and graphite. Doped oxides such as indium tin oxide, and semiconductors such as silicon oxide and gallium arsenide are also contemplated. Additionally, the electrodes may be composed of conducting polymers, metal doped polymers, conducting ceramics and conducting clays. Among the noble metals, platinum and palladium are especially preferred because of the advantageous properties associated with their ability to absorb hydrogen, i.e., their ability to be "preloaded" with hydrogen before being used in the methods of the invention.

In accordance with other preferred embodiments of the present invention, one or more of the electrodes are proximate to a "getter" structure. Preferably the "getter" structure comprises a second electrode. The second electrode may be of any shape or size. However, it may function to scavenge electrochemically generated reagents alone or in conjunction with a scavenging solution and/or a buffering solution or it may function to reduce or eliminate diffusion of ions into nearby electric sources such as semiconductor circuitry. Such second electrodes may be made of the same material as the selected electrodes discussed above.

The electrode(s) used in accordance with the invention may be connected to an electric source in any known manner. Preferred ways of connecting the electrodes to the electric source include CMOS switching circuitry, radio and microwave frequency addressable switches, light addressable switches, and direct connection from an electrode to a bond pad on the perimeter of a semiconductor chip. The placement of a "getter" structure in accordance with the description set forth above and such as the structure exemplified in FIGS. 31 and 34–36 effectively prolongs the life of a semiconductor chip thereby making such a connection particulary advantageous.

CMOS switching circuitry involves the connection of each of the electrodes to a CMOS transistor switch. The switch is accessed by sending an electronic address signal down a common bus to SRAM (static random access memory) circuitry associated with each electrode. When the switch is "on", the electrode is connected to an electric source. This is a preferred mode of operation.

Radio and microwave frequency addressable switches involve the electrodes being switched by a RF or microwave signal. This allows the switches to be thrown both with and/or without using switching logic. The switches can be tuned to receive a particular frequency or modulation frequency and switch without switching logic. Alternatively, the switches can use both methods.

Light addressable switches are switched by light. In this method, the electrodes can also be switched with and without switching logic. The light signal can be spatially localized to afford switching without switching logic. This is accomplished, for example, by scanning a laser beam over the electrode array; the electrode being switched each time the laser illuminates it. Alternatively, the whole array can be flood illuminated and the light signal can be temporally modulated to generate a coded signal. However, switching logic is required for flood illumination.

One can also perform a type of light addressable switching in an indirect way. In this method, the electrodes are formed from semiconductor materials. The semiconductor electrodes are then biased below their threshold voltage. At sufficiently low biases, there is no electrochemistry occurring because the electrons do not have enough energy to overcome the band gap. The electrodes that are "on" will already have been switched on by another method. When the electrodes are illuminated, the electrons will acquire enough energy from the light to overcome the band gap and cause electrochemistry to occur.

Thus, an array of electrodes can be poised to perform electrochemistry whenever they are illuminated. With this method, the whole array can be flood illuminated or each electrode can be illuminated separately. This technique is useful for very rapid pulsing of the electrochemistry without the need for fast switching electronics. Direct connection from an electrode to a bond pad on the perimeter of the semiconductor chip is another possibility, although this method of connection could limit the density of the array.

Electrochemical generation of the desired type of chemical species requires that the electric potential of each electrode have a certain minimum value. That is to say, a certain minimum potential is necessary, which may be achieved by specifying either the voltage or the current. Thus, there are two ways to achieve the necessary minimum potential at each electrode: either the voltage may be specified at the necessary value or the current can be determined such that it is sufficient to accommodate the necessary voltage. The necessary minimum potential value will be determined by the type of chemical reagent chosen to be generated. One skilled in the art can easily determine the necessary voltage and/or current to be used based on the chemical species desired. The maximum value of potential that can be used is also determined by the chemical species desired. If the maximum value of potential associated with the desired chemical species is exceeded, undesired chemical species may be resultantly produced.

The substrates prepared in accordance with the present invention will have a variety of uses including, for example, screening large numbers of polymers for biological activity. To screen for biological activity, for example, in the field of pharmaceutical drug discovery, the substrate is exposed to one or more receptors such as antibodies, whole cells, receptors on vesicles, lipids, or any one of a variety of other receptors. The receptors are preferably labeled with, for example, an electrochemical marker, an electrochemiluminescent marker, a chemiluminescent marker, a fluorescent marker, a radioactive marker, or a labeled antibody reactive with the receptor. The location of the marker on the substrate is detected with, for example, electrochemical, fluorescence or autoradiographic techniques. Through knowledge of the sequence of the material at the location where binding is detected, it is possible to determine quickly which sequence binds with the receptor and, therefore, the technique can be used to screen large numbers of peptides.

The present invention can also be used for therapeutic materials development, i.e., for drug development and for biomaterial studies, as well as for biomedical research, analytical chemistry and bioprocess monitoring. An exemplary application of the present invention includes diagnostics in which various ligands for particular receptors can be placed on a substrate and, for example, blood sera can be screened. Another exemplary application includes the placement of single or multiple pre- formed receptor molecules at selected sites on a substrate and, for example, drug screening could be conducted by exposing the substrate to drug candidate molecules to determine which molecules bind to which pre-formed receptor molecules.

Yet another application includes, for example, sequencing genomic DNA by the technique of sequencing by hybridization. Another contemplated application includes the synthesis and display of differing quantities of molecules or ligands at different spatial locations on an electrode array chip and the subsequent performance of dilution series experiments directly on the chip. Dilution series experiments afford differentiation between specific and non-specific binding of, for example, ligands and receptors. Non-biological applications are also contemplated, and include the production of organic materials with varying levels of doping for use, for example, in semiconductor devices. Other examples of non-biological uses include anticorrosives, antifoulants, and paints.

It is specifically contemplated that the present invention may be used for developing materials. Materials may be developed by methods according to the present invention for many purposes including, but not limited to corrosion resistance, battery energy storage, electroplating, low voltage phosphorescence, bone graft compatibility, resisting fouling by marine organisms, superconductivity, epitaxial lattice matching, or chemical catalysis. Materials for these or other utilities may be formed proximate to one or a plurality of electrodes. Alternatively, materials may be formed by modifying the surface of one or a plurality of electrodes by generating reagents electrochemically. Additionally, materials may be formed by modifying the bulk electrode material of one or a plurality of electrodes using electrochemically generated reagents.

It is further contemplated that methods according to the present invention may be used to develop protocols for testing materials. That is, reagents electrochemically generated by methods according to the present invention may be used to test the physical and chemical properties of materials proximate to one or a plurality of electrodes. For instance, skilled artisans may readily develop protocols to evaluate such properties as corrosion resistance, electroplating efficiency, chemical kinetics, superconductivity, electrochemiluminescence and catalyst lifetimes using electrochemically generated reagents in accordance with the present invention.

The present invention will further be clarified and illustrated by the following examples, which are intended to be merely exemplary of the invention.

EXAMPLES

Example 1

Combinatorial Synthesis of the Leu-enkephalin epitope

Background

Endorphins are naturally occurring small peptides (including approximately 20–40 amino acids) that bind to opiate receptors in the brain. It has been discovered that most of the activity of endorphins is due to the last five amino acids on the peptides. These terminal pentapeptides are called enkephalins.

The immunofluorescent technique for detecting the Leu-enkephalin epitope follows standard detection protocols. See for example, F. M. Ausubel et al., *Short Protocols in Molecular Biology*, Third edition, Unit 14, pgs. 14–23ff (1995). This assay requires a primary antibody, e.g., the 3-E7 monoclonal antibody, and a secondary antibody-fluorochrome conjugate specific to the source species of primary antibody, e.g., the goat anti-mouse fluorescent conjugate. The 3-E7 antibody is a mouse monoclonal antibody against endorphins that bind to leu-enkephalins. Both of the antibodies for this technique can be obtained from Boehringer Mannheim Biochemicals, Indianapolis, Ind.

For additional information regarding the 3-E7 monoclonal antibody, see, e.g. Meo, Tommaso, et al., "Monoclonal antibody to the message sequence TryGly-Gly-Phe of opioid peptides exhibits the specificity requirements of mammalian opioid receptors," *Proc. Natl. Acad. Sci USA* 80, pps. 4084–4088 (1983).

Preparation of an electrode array for use in combinatorial synthesis

An 10×10 platinum electrode array is used, as is shown in FIG. 6. Columns 1 and 10 are used as counter electrodes. The active columns of the array are columns 2,3,5,6 and 7. Columns 4,8 and 9 are never activated in this synthesis.

The surface of the array is modified with a permeable membrane layer formed from controlled porosity glass (CPG) that is applied to the array by deposition of silicon dioxide under appropriate conditions in the semiconductor manufacturing process. The CPG forms a chemically inert membrane that is permeable to ions. This membrane is functionalized by silanation with chloromethyl silane. The chloromethyl silane groups are further modified by ethylene glycol linker molecules containing ten ethylene glycol moieties by reacting the silanized CPG membrane with a molecule containing ten ethylene glycol moieties and two amino groups at each end. This membrane provides a layer overlaying the surface of the array that is functionalized by amine groups that are, in turn, attached to the CPG matrix via a silane moiety. The diamino ethylene glycol molecules act as linker molecules (spacer groups) between the membrane and the epitope molecules which are formed.

Addition of protected functional groups to the membrane

The functionalized CPG membrane covered electrode array is exposed to a DMF solution of benzyloxycarbonyl (CBZ) protected 1-leucine containing coupling reagents, such as, but not limited to, dicyclohexylcarbodiimide (DCC) or diisopropylcarbodiimide, at room temperature for approximately two hours. This exposure produces a CPG membrane layer covering the array that is completely covered with CBZ-protected 1-leucine moieties attached to the membrane layer by ethylene glycol linker molecules. This moiety covered membrane layer is shown in FIG. 7. This is the bed of molecules on which the epitope molecule is built.

The moiety covered membrane layer is then washed three times with an aqueous 0.1 M phosphate buffer solution having a pH of 7.4.

Removal of the protecting groups (deprotection)

Removal of the CBZ protecting groups from the protected amino acids, i.e., deprotection, using electrochemically generated reagents (protons) is performed as follows.

Referring to the electrode array of FIG. 6, a preconditioning step is performed: columns 2, 3, 5, 6, and 7 are biased negative with respect to columns 1 and 10, which serve as counter electrodes. There is no reference electrode in this system. The potential difference is approximately 3 volts, which voltage is applied for approximately 10 seconds. This preconditioning step causes hydroxyl ions to be formed at the electrodes with a negative bias and protons to be formed at the counter electrodes having a positive bias. This preconditioning step also causes protons to be reduced to hydrogen molecules at electrodes with a negative bias. The platinum electrodes absorb and hold some of these hydrogen molecules in the bulk metal.

Following the preconditioning step, the bias is then reversed. The electrodes of columns 1 and 10 (counter

electrodes) are biased negative with respect to columns 2, 3, 5, 6, and 7. The potential difference is approximately 2.6 volts, which voltage is applied for approximately three seconds. This step causes protons to be formed at the electrodes with a positive bias both from hydrolysis of water and from oxidation of hydrogen molecules that are absorbed into the platinum electrodes during the preconditioning step. As a result of the preconditioning step and this subsequent step, the CBZ protecting groups are removed from the leucine amino acid moieties at the electrodes in columns 2,3, 5,6, and 7.

These two steps result in deprotected reactive amine moieties remaining attached to the leucine molecules at these sites (columns 2, 3, 5, 6, and 7) as illustrated in FIG. 8.

Preparation of the membrane for coupling

To prepare the reactive amine moiety covered membrane for coupling CBZ-L-phenylalanine to the deprotected leucine groups, the following steps are performed:

The electrode array containing the reactive amine moiety covered membrane is washed twice with pure DME. The electrode array is then exposed to a DMF solution containing CBZ-L-phenylalanine and coupling reagents, such as DCC at room temperature for approximately two hours. This step results in the electrodes of columns 2, 3, 5, 6, and 7 being modifed with an CBZ-protected dipeptide of leucine and phenylalanine. This is shown in FIG. 9.

The deprotection and coupling steps are then repeated at columns 3, 5, 6, and 7. That is, the electrode array is again exposed to an aqueous 0.1 M phosphate buffer solution having a pH of 7.4. The electrode array is then exposed to a DMF solution of CBZ- protected glycine and coupling reagents for approximately 2 hours at room temperature. This results in the electrodes in columns 3, 5, 6, and 7 being modified with the CBZ- protected tripeptide glycine-phenylalanine-leucine (G-F-L), as shown in FIG. 10.

The deprotection and coupling steps are then repeated at columns 5, 6, and 7. That is, the electrode array is again exposed to an aqueous 0.1 M phosphate buffer solution having a pH of 7 4 and then exposed to a DMF solution of CBZ-protected glycine and coupling reagents for approximately two hours at room temperature. This results in the electrodes in columns 5, 6, and 7 being modified with the CBZ- protected tetrapeptide glycine-glycine-phenylalanine-leucine (G-G-F-L).

The deprotection and coupling steps are then repeated at columns 6 and 7 while the electrode array is again exposed to an aqueous 0.1 M phosphate buffer solution having a pH of 7.4. The electrode array is then exposed to a DMF solution of CBZ-protected l-tyrosine and coupling reagents for approximately two hours at room temperature. This results in the electrodes in columns 6 and 7 being modified with the CBZ-protected pentapeptide tyrosine-glycine-glycine-phenylalanine-leucine (Y-G-G-F-L), as shown in FIG. 11. This is the CBZ-protected version of the desired Leu-enkephalin epitope.

The deprotecting step is then repeated at columns 2, 3, 5, and 6, without a preconditioning step, to remove the CBZ protecting groups from the terminal amino acids of the combinatorial sequences. This procedure produces the following sequences:

Columns 1 and 10: modified with the protected Leu-enkephalin epitope (these are the counter electrodes).

Column 2: modified with the deprotected dipeptide F-L.

Column 3: modified with the deprotected tripeptide G-F-L.

Columns 4,8 and 9: modified with the CBZ-protected leucine amino acid.

Column 5: modified with the deprotected tetrapeptide G-G-F-L.

Column 6: modified with the deprotected Leu-enkephalin epitope.

Column 7: modified with the CBZ-protected Leu-enkephalin epitope.

3-E7 monoclonal antibody assay

The modified electrode array, i.e., with the 10 modified columns, is exposed, via the Leu-enkephalin epitope detection technique discussed above, to the 3-E7 monoclonal antibody, followed by exposure to the goat anti-mouse fluorescent conjugate. The electrode array is then examined using an epifluorescent microscope. The expected results are shown in FIGS. 12 and 13. As is shown in FIGS. 12 and 13, the active Leu-enkephalin epitope is present proximate to the electrodes of column 6. (Column 6 is the only column modified with the deprotected Leu-enkephalin epitope.)

Note: The synthesis proceeds at the counter electrodes (electrodes 1 and 10) because protons are generated at the counter electrodes during each preconditioning (deprotecting) step. Since a preconditioning step is not performed in the final deprotection step, no protons are produced at the counter electrodes in the final step and a protected Leu-enkephalin epitope is produced at the counter electrodes, which does not react upon exposure to the antibody and fluorescent conjugate.

Example 2

Combinatorial Synthesis of Deoxyribonucleic acids

Background

The monomer units for combinatorial synthesis of DNA are called phosphoramidites. Phosphoramidites are linked together into a single strand nucleic acid polymer through phosphodiester bonds. Since the phosphorous is protected by a cyanoethyl ether moiety during synthesis, the bonds are phosphotriester bonds. The cyanoethyl group can be removed by a base at the end of synthesis to give the phosphodiester linkage. Phosphoramidites have two ends that are called 3' and 5' ends. The 3' end of one phosphoramidite will couple with the 5' end of another. Usually the 3' end is attached to a solid support and the 5' end is modified by another phosphoramidite to start the synthesis cycle. The 5' end is a hydroxy group that can be protected by a molecule called dimethyltrityl (DMT). DMT groups are acid labile protecting groups.

There are four naturally occurring deoxyribonucleotide monomers that form DNA polymers. They are adenosine (A), thymidine (T), cytosine (C), and guanosine (G). DNA is considered an acid because the phosphodiester groups that bind the monomers together are acidic. The nucleosides (A,T,C, G) are organic bases. DNA in nature is normally tens of millions to billions of base units long. A fifteen base unit long piece of DNA will be prepared in the following example. A piece of DNA of this length is known as a oligonucleotide. DNA molecules should be at least this long, otherwise it is very difficult to distinguish between them.

The nucleosides are protected because the exocyclic amine bases (A, C, G) are susceptible to depurination by acids. The protecting groups on these bases are base labile. There are three kinds of protecting groups on phosphoramidites. They are the DMT groups, which protect the 5' hydroxyl groups, the cyanoethyl ether groups, which protect the phosphorous, and the FOD (fast oligonucleotide

deprotection) groups, which protect the exocyclic amines on the nucleoside bases. The DMT groups are acid labile and the others are base labile.

DNA is found in nature mostly as the "duplex" form having the famous double helix structure. This means that two single strands of DNA are bound together by interactions between the nucleoside bases. The nucleoside base T interacts with the nucleoside base A to form an A-T linkage. The nucleoside base C interacts with the nucleoside base G to form a C-G linkage. The A-T and the C-G interactions are the only stable interactions; other combinations are weak. Linkages that are not A-T or C-G can occur, and are called mismatches. When two complimentary single strands of DNA come together to form a duplex, this is called hybridization. When the single strands of DNA in a duplex come apart, the duplex DNA is said to have denatured. DNA duplexes typically denature when they are exposed to heat and/or low ionic strength aqueous solutions.

To determine whether or not a specific DNA sequence has been synthesized at a particular site, one uses probe strands of DNA that are complimentary with the strands that presumably were synthesized at that site. These probe strands are labeled covalently with a fluorescent dye. The probe strands will bind to DNA molecules on the surface with both the correct sequence and the incorrect sequence. However, the melting temperatures are much lower for the DNA duplexes that contain mismatches, i.e., non A-T and C-G link, than those that are complimentary, i.e., A-T and C-G links. Thus, upon heating, the probes forming duplexes with the incorrect DNA strands win denature first. By increasing the temperature to a level where all of the mismatched DNA duplexes have denatured, it is possible to detect only the DNA molecules with the correct sequence by observing the fluorescent dye using epifluorescent microscopy. Alternatively, the test surface can be washed with low ionic strength aqueous solutions. This has the same effect as raising the temperature and is more convenient experimentally.

Synthesis procedure

The electrode array is first modified with an acrylate/polyvinyl alcohol copolymer layer or membrane. The copolymer layer contains numerous pendant hydroxyl groups that are reactive toward phosphoramidites. The polymer modified electrode array is then exposed to DMT-protected cytidine phosphoramidite and tetrazole at a concentration of 0.05 M in an anhydrous acetonitrile for 30 seconds at room temperature. The cytosine base and all of the other bases used in this example are protected using the FOD protecting scheme. (FOD protecting groups afford the best protection against depurination of exocyclic amines.) The array is then washed with anhydrous acetonitrile. Any unreacted hydroxyl groups on the surface are then capped by exposing the surface to an anhydrous acetonitrile solution of acetic anhydride and 1-methylimidizole for thirty seconds. This results in a surface modified everywhere with DMT protected C base units.

The trivalent phosphite linkage between the polymer and the phosphoramidite is oxidized to the more stable pentavalent phosphotriester linkage by electrochemically generated iodine. The iodine is produced electrochemically by the oxidation of iodide ions in an aqueous THF solution of potassium iodide. Iodine can be confined to the local area where it is formed by both an iodine buffering reaction and a scavenging reaction. Iodine is buffered by an equilibrium reaction with iodide ions to form the triiodide ion. The triiodide ion is not a useful reagent. Further, the solution can be buffered with respect to hydroxyl ions such that it is slightly basic. Iodine reacts with hydroxyl ions to form iodide ions and hypoiodite. Both of these chemical species are unreactive. Thus, hydroxyl ions serve as scavengers for iodine. Because the electrochemical oxidation of iodide ions to iodine can occur under conditions that also produce protons, the local environment can be made acidic while the iodine is being generated. There will be no scavenging in the acidic regions where iodine needs to be active. As a result, there are stable phosphodiester linkages to the polymer film only over those electrodes that electrochemically generate iodine. The unoxidized phosphite linked groups will eventually fall off after repeated exposure to the acetic anhydride capping solution.

The electrode array is next exposed to an aqueous 0.1 M sodium phosphate solution. A positive potential is applied for one second to first selected areas and the DMT protecting groups are removed from the cytidine phosphoramidites in first selected areas. The array is then washed with anhydrous acetic anhydride. The reactive array is then exposed to a 0.05 M solution of thymidine phosphoramidite, T, and tetrazole in anhydrous acetonitrile for 30 seconds. The T nucleotides react with the C nucleotides at the first selected sites to form a C-T dimer. The remaining unreacted C nucleotides are capped and the phosphite linkages are reduced to phosphotriester linkages as outlined above.

This procedure is repeated at second, third, fourth, and so on, selected sites to synthesize combinatorially four different fifteenmer oligonucleotides at selected sites on the array. The array is then exposed to a 0.1 M aqueous ammonium hydroxide solution at 50° C. for an hour. The FOD protecting groups and the cyanoethyl protecting groups on the phosphotriester are removed by the hydroxyl ions. The resulting array consists of single strands of the oligomer nucleic acids bound covalently to the polymer membrane.

Evaluation of the fidelity of the array

The fidelity of the combinatorial array is tested using four different fluorescently labeled oligonucleotide probes that are complimentary to the oligonucleotides synthesized on the array. The array is exposed to a first 100 nanomolar solution of a fluorescently labeled oligonucleotide probe in a 0.1 M sodium phosphate buffer at pH 7.2 at room temperature for thirty minutes. The array is then washed three times with a 0.1 M sodium phosphate buffer solution at pH 7.2. The array is then examined with an epifluorescent microscope. Bright spots appear in first areas where the oligonucleotide probe is present To ensure that the oligonucleotide probe and its compliment actually hybridized, the array is washed several times with deionized water at 70° C. for five minutes. Reexamination of the array with the epifluorescent microscope reveals a dark field. This means that the probe hybridized to its compliment and the results are not due to nonspecific absorption. The array is then exposed to a second 100 nanomolar solution of another fluorescently labeled oligonucleotide probe in 0.1 M aqueous sodium phosphate buffer at pH 7.2. The array is subsequently washed, examined with the epifluorescent microscope and then checked for nonspecific absorption. Bright spots appear in the second areas where the nucleotide probes are synthesized. The procedure is repeated for the third and fourth oligonucleotide sequences. The control areas will not bind the fluorescently labeled probe and become bright at any point in the assay.

Example 3 and Comparative Example 4

For the following example and comparative example, results were recorded and reproduced in the form of video

photomicrographs that were captured digitally of the respective electrode array chips under various conditions.

Recording of Results - Taking of Pictures

The photomicrographs were taken using an Olympus *BX60 microscope with a Pulnix TM-745 integrating CCD* camera. The camera was controlled by, and the images were captured by, a Data Translation DT3155 video capture card run by a Pentium-based personal computer. The software that controlled the DT3155 card can easily be written by one of ordinary skill in the art.

Most of the photomicrographs were taken with a 10x objective that allowed approximately 16 electrodes to be seen in each image; however, for purposes of evaluation, the images were sometimes cropped to focus on the activity of the electrodes of interest. At times, a 4x objective was also used. Two types of photomicrographs were taken. A few were taken using white light illumination. In these, the electrodes appear reflective. For example, see FIG. **14**. The majority of the photomicrographs were taken using epifluorescent illumination. In these, the electrodes appear dark in the photomicrographs when they are uncoated, i.e., when no fluorescent coating is present, because the metal of the electrodes, e.g., the platinum, quenches any fluorescence present.

Epifluorescent microscopy involves illuminating the electrode array chip from a position above the chip surface, along a path normal to the chip surface. The illuminating beam is filtered to obtain a narrow band centered at the excitation wavelength of the fluorescent dye being used. The fluorescent dye used in the following example and comparative example was Texas Red, which has an absorption maximum at 595 nm. This dye emits a fluorescent light with an emission maximum at 615 nm when it is excited with light of approximately 595 nm. Texas Red can be obtained from Molecular Probes, Eugene Oreg. Filters in the Olympus BX60 microscope prevent the excitation light from traveling to the optical detector of the CCD camera. The Olympus BX60 microscope is equipped with an ancillary art-recognized instrumentation module to perform epifluorescent microscopy using Texas Red dye.

Exemplary photomicrographs taken using white illumination and epifluorescent illumination are shown in FIGS. **14–16**. FIGS. **14** and **15** depict an uncoated electrode array chip, while FIG. **16** depicts an electrode array chip coated with a fluorescent membrane.

Description and Preparation of the electrode array chips

The chips prepared and used in the following example and comparative example were rectangular devices with a 16 (in the x-direction) by 64 (in the y-direction) array of 100 micron diameter platinum electrodes. The total number of electrodes in these arrays was 1024. The dimensions of the chips were approximately 0.5 cm (x- direction) by 2.3 cm (y-direction), and the total surface area of the chips was approximately 1 square centimeter. The electrodes in each array were approximately 250 microns apart in the x-direction and approximately 350 microns apart in the y-direction, measured from the center of the electrodes.

Each electrode in the array was capable of being addressed independently using an SRAM cell (static random access memory), a standard art-recognized way to address independently electric circuitry in an array. The SRAM cell was located next to the electrodes in the electrical circuitry associated with electrode. Each electrode in the array had four separate switchable voltage lines that attached to it, allowing each electrode in the array to be switched independently from one voltage line to another. The voltage was arbitrary and was set by an external voltage source.

In the chips used in the following example and comparative example, there were additionally 13 electrodes on the side of the chips that were hard wired to bond pads, meaning they were not switchable or independently addressable as were the electrodes in the 16x64 array. These 13 electrodes had no circuitry associated with them except for a single voltage line, and thus allowed protocols to be run-on them without engaging the associated electrode array. These 13 electrodes were 100 microns in diameter and were spaced differently from the electrodes in the array. See, for example, FIG. **17**, showing the triangular orientation of the hard-wired electrodes, wherein the electrodes are 250 microns apart from the centers of the electrodes.

The chips were made by a 3 micron process using hybrid digital/analog very large scale integration (VLSI). One skilled in the art would be familiar with such a process and could easily prepare a chip for use in accordance with the present invention. See, Mead, C., *Analog VLSI and Neural Systems*, Addison/Wesley (1989). The circuitry used was CMOS (complimentary metal-oxide silicon) based and is also well known to those of ordinary skill in the art.

The chips were controlled by at least one Advantech PCL-812 digital I/O card (in the computer) that was driven by a Pentium based personal computer. These digital I/O cards can be obtained from Cyber Research, Branford, Conn. Preferably the chip is connected through interface hardware, i.e., an interface card, to the I/O card. The software for driving the I/O card can easily be written by one of ordinary skill in the art. DC voltage for powering the chips was provided by the PCL-812 and/or a Hewlett-Packard E3612A DC power supply. Voltage for the electrodes was supplied by the PCL-812 card and/or by an external Keithley 2400 source- measure unit.

The electrode array chips were designed so that the bond pads for all of the on- chip circuitry were located at one end of the long side of the chips. See FIGS. **18***a* and **18***b*. The chips were attached to a standard 121 pin PGA (pin grid array) package that had been sawn in half so that approximately 2 cm of the chip extended out from the end, analogous to a diving board. See FIG. **18***b*. PGA packages can be obtained from Spectrum Semiconductor Materials, San Jose, Calif. Connecting wires ran between the bond pads on the chip and the contacts (bond pads) on the PGA package. The bond pads on the chip, the connecting wires, and the contacts on the PGA package were covered with epoxy for protection and insulation. See cut away in FIG. **18***a*. The section of the chips that extended into the air contained the electrode array and was not covered by epoxy. This section of the chips was available for dipping into solutions of interest for chemical synthesis at the electrodes at the surface of the chip. One of ordinary skill in the art could easily set up and design chips appropriate for use in accordance with the present invention.

Example 3 (Inventive) - Deprotection and Localization

Background Description

One of the above described electrode array chips comprising 16×64 platinum electrodes was used for this example. As indicated above, the chip contained 13 hard-wired electrodes located at one end of the long side of the chip, however, these hardwired electrodes were not involved in this example.

The model chemical system used in this example to demonstrate localization and selective deprotection using electrochemically generated reagents involved attaching fluorescent labeled streptavidin molecules, a well-known

variety of avidin, obtainable from Vector Laboratories, Burlingame, Calif., to a membrane overlaying the electrode array chip via a trityl linker molecule. The overlaying membrane used was polysaccharide-based. The trityl linker molecule used was acid labile, i.e., labile to protons, and detached from the overlaying membrane in the presence of protons, taking with it the attached fluorescent labeled streptavidin molecule. More specifically, the trityl linker molecule used was a modified 4,4'-dimethoxytrityl molecule with an exocyclic active ester obtained from Perseptive Biosystems, Framingham, Mass.

Experimental Procedure

Preparation of the chip for attachment of molecules

To enable the attachment of molecules, in particular trityl linker molecules, to the surface of the electrode array chip for synthesis and/or deprotection proximate the electrodes, the chip was coated/modified with an overlaying membrane of a polysaccharide-based material. Specifically, a polygalactoside was used as the overlaying membrane material in this example. The polygalactoside membrane was dip coated onto the chip. However, dipping or coating according to any method known to one of ordinary skill in the art would be acceptable.

Attachment of the trityl linker molecules

Once the electrode array chip was coated with the polysaccharide membrane, the trityl linker molecules were attached to the chip. The trityl linker molecule used for this example was a modified 4,4'-dimethoxytrityl molecule with an exocyclic active ester, specifically the molecule was N-succininimidyl-4[bis-(4-methoxyphenyl)-chloromethyl]-benzoate. The synthesis and use of this molecule is described in *A Versatile Acid-Labile Linker for Modification of Synthetic Biomolecules*, by Brian D. Gildea, James M. Coull and Hubert Koester, *Tetrahedron Letters*, Volume 31, No. 49, pgs 7095–7098 (1990).

The trityl linker molecules were attached to the polysaccharide membrane via immersion of the polysaccharide membrane coated chip in a DMF solution containing 0.5M of tertbutyl ammonium perchlorate, 0.75M of 2,4,6-collidine and 0.2M of the trityl linker. The immersion of the polysaccharide membrane coated chip in the DMF linker solution lasted for 30 minutes at ambient temperature. The trityl linker coated chip was then washed with DMF to remove any remaining reactants. Next, the trityl linker coated chip was washed in an aqueous 0.1 M sodium phosphate buffer that was adjusted to pH 8.0, and dried.

Attachment of the fluorescent dye labeled molecules

The trityl linker coated chip was then immersed in an aqueous solution of fluorescent dye (Texas Red) labeled streptavidin molecules having a concentration of 50 micrograms per milliliter and allowed to remain in this solution for one hour at ambient temperature. During this immersion, the linker molecule was derivatized and the fluorescent dye labeled streptavidin molecules were attached to the linker molecules.

The chip containing fluorescent dye labeled streptavidin molecules was then washed with an aqueous 0.1M sodium phosphate buffer that was adjusted to pH 8.0 to remove remaining reactants, and dried. The chip was now ready for use in the electrochemical process of the invention, i.e., the selective deprotection step.

Following exposure of the prepared chip to the fluorescent labeled streptavidin molecules, but prior to any electrical current or voltage being applied, the electrodes in the array were all bright with fluorescence because the membrane proximate to them contained the fluorescent labeled streptavidin molecules bound to the membrane via the trityl linker. A photomicrograph of this is shown in FIG. 19*a*.

Selective Deprotection

To perform the selective deprotection step, the prepared chip was immersed in a 0.05M aqueous sodium phosphate buffer solution to enable electrochemical generation of reagents. A voltage difference of 2.8 volts was applied to select electrodes (alternating in a checkerboard pattern) for approximately 10 minutes, causing protons to be generated electrochemically at the anodes.

After the protons were electrochemically generated at the anodes, the anodes became dark because the trityl linker previously bound proximate to. the anodes dissociated from the anodes and the fluorescent labeled streptavidin molecules were washed away. The extent to which this occurred at the anodes and not at the cathodes in the checkerboard pattern, is a measure of the chemical crosstalk occurring between the electrodes in the array. That is, if chemical crosstalk were occurring, the cathodes would also be dark because the protons would have migrated and dissociated the trityl linkers at the cathodes.

Thus, under epifluorescent microscopy, the bright electrodes (cathodes) indicate the presence of a Texas Red labeled streptavidin molecule bound to a linker molecule at the electrode and the dark electrodes (anodes) indicate the lack of a Texas Red labeled streptavidin molecule bound to a linker molecule at the electrode. This is shown in FIGS. 20 and 21, FIG. 20 having been taken using a 4× objective with an integration time of 2 seconds, and FIG. 21 having been taken using a 10× objective with a 500 millisecond integration time.

Results

Following drying of the chip, photomicrographs were taken of the electrode array following completion of the deprotection step, and are reproduced in FIGS. 20 and 21. As shown in these figures, selective deprotection was achieved using the process of the present invention. As is shown in these figures, a repeating checkerboard pattern was produced, exemplifying that the process of the present invention achieved localization of the protons generated at the anodes and prevented migration of these protons to the cathodes. The dark areas (anodes) are clearly defined and distinguished from the also clearly defined bright areas (cathodes). The clearly demarcated checkerboard pattern shown in the photomicrographs indicates that no, or very little, chemical cross talk occurred during the deprotection step.

Example 4

Comparative Example

Using two electrode array chips prepared in accordance with the present invention, one chip was processed using the selective deprotection procedure in accordance with the present invention using a buffering solution, and the second chip was processed using a selective deprotection procedure varying only in that the electrolyte used in the Examples of Southern (WO 93/22480, held Nov. 11, 1993) replaced the buffering solution of the present invention.

Rather than using an electrode array, this comparison was conducted on a few of the hard wired electrodes found on the side of the electrode array chips. FIG. 17 is a photomicrograph taken under the same conditions as FIG. 14, but showing the hard wired electrodes used in this example.

Deprotection in accordance with the invention

The steps of coating the chip with the polysaccharide membrane and attaching the trityl linker molecules to the membrane were performed in accordance with the procedures used above in Example 3.

The attaching of the fluorescent dye labeled streptavidin molecules and the deprotection steps were also performed in accordance with Example 3, but a 20 mM aqueous sodium phosphate buffer solution was used instead of the 0.05M solution used in Example 3, to enable the electrochemical generation of reagents. The voltage that was applied between selected electrodes was 2.8 volts, which was applied for approximately 30 seconds.

Similar results to Example 3 were obtained. These results are shown in FIGS. 22–24.

FIG. 22 shows the hardwired electrodes involved in this process, labeled as T1, T2 and T4. In this process, T1 was the counter electrode, i.e., the cathode, and T2 and T4 were the anodes where protons were generated upon the application of the electric current or voltage. No voltage had been applied to the electrodes shown in FIG. 22.

FIG. 23 shows the same electrodes following derivatization or bonding with the fluorescent labeled streptavidin molecules. As is shown, electrodes T2 and T4 are bright, indicating the presence of a Texas Red labeled streptavidin molecule bound to a linker molecule proximate each of these electrodes.

FIG. 24 shows the condition of anodes T2 and T4 following application of the voltage causing electrochemical generation of protons at the anodes and resultant dissociation of the trityl linker at these positions. Once dissociation occurred, the fluorescent labeled streptavidin molecules were washed away, leaving the anodes dark. Notably, anodes T2 and T4 are darker than the neighboring electrodes, indicating no chemical crosstalk was occurring.

As is shown by FIGS. 23 and 24, localization and selective deprotection were achieved at anodes T2 and T4, as was desired.

Deprotection using electrolyte of Southern (WO 93/24480)

All steps were performed identical to that for the above process in accordance with the present invention, except that instead of using a buffering solution in accordance with the invention, deprotection was performed in the presence of a 1% triethylammonium sulfate electrolyte in an acetonitrile solvent, as disclosed in the Examples of Southern.

The results of this process are shown in FIGS. 25*a*, 25*b*, 26*a* and 26*b*. In the electrodes shown, labeled T1 and T4, electrode T1 represented the cathode and electrode T4 represented the anode.

FIGS. 25*a*, 25*b*, 26*a* and 26*b* show that the membrane exhibited random and imprecise bright and dark areas. These bright and dark areas indicate that the protons generated at the anode (electrode T4) are not confined or localized to the area proximate the electrode, causing significant dissociation of the trityl linker over the entire field of the photomicrograph T1 appears to have retained most of the fluorescence directly above the electrode. This is explained by the base that is generated at the T1 cathode, which neutralized the acid generated proximate the T4 anode.

As is seen from a comparison of the photomicrographs illustrating the results achieved in accordance with the present invention (i.e., using a buffering solution overlaying the electrodes) and those illustrating the results achieved from the analogous experiment performed using the electrolyte of Southern (WO 93/22480), superior localization of the electrochemical generated reagents was achieved using the process of the present invention. The superior localization achieved in accordance with the present invention greatly reduced, if not eliminated, undesirable chemical crosstalk between proximate electrodes. In contrast, very little localization of the electrochemical generated reagents was achieved using the electrolyte of the prior art, resulting in random and imprecise deprotection over the entire field of the micrograph.

Example 5

Formation of Carbon—Carbon Bonds by Electrochemically-Catalyzed Olefin Addition Reactions.

Microscopy and chip control were performed according to the descriptions set forth in Examples 3 and 4 described above.

Electrode array chips comprising 16x64 platinum electrodes with thirteen ancillary hardwired electrodes were used in this example. Electrochemistry was conducted on a few of the hardwired electrodes found on the side of the array in this example. This example demonstrates the formation of carbon—carbon bonds by the method of this invention between an activated olefin immobilized proximate to one or a plurality of electrodes and an anhydride contained in a solution to which the one or a plurality of electrodes is exposed. The overlaying membrane was polysaccharide-based. The activated olefins were attached covalently to the overlaying membrane. More specifically, the activated olefins were acryloyl groups. The anhydride contained in the solution was a biotin anhydride.

An electrochemically activated catalyst was used to mediate the coupling of biotin to the immobilized olefins. More specifically vitamin B_{12} was used as the catalyst. The active component of vitamin B_{12} is a cobalt atom. Normally, the formal oxidation state of the cobalt atom in vitamin B_{12} is +3 (Co(III)). Vitamin B_{12} can be reduced electrochemically such that the formal oxidation state of the cobalt atom is +1(Co(I)). The Co(I) species is active as a catalyst that mediates the formation of carbon—carbon bonds.

Experimental Procedure

Preparation of bulk acryloyl modified polysaccharide materials.

Acryloyl groups were added to hydroxyl moieties on polysaccharides by the following procedure. A mixture of 0.2 g of the bulk polysaccharide, 0.05 g of acryloyl chloride (Aldrich, Milwaukee, Wis.), 0.1 ml of pyridine (Aldrich, Milwaukee, Wis.) in 5 ml of DMF (Aldrich, Milwaukee, Wis.) was stirred at room temperature for 30 minutes. The suspended polysaccharide was isolated by filtration. The acryloyl-modified polysaccharide was washed with DMF, then deionized water, and then acetone. The washed filter cake was dried in vacuo overnight at room temperature.

Preparation of biotin anhydride.

A stirred suspension of 0.09 g of d-biotin (Sigma, St. Louis, Mo.) in 5 ml of dry THF (Aldrich, Milwaukee, Wis.) was degassed with dry nitrogen. 30 microliters of thionyl chloride (Aldrich, Milwaukee, Wis. was added dropwise to the stirred suspension. The reaction mixture was stirred under nitrogen at room temperature for one hour. All of the suspended d-biotin went into solution upon reaction. The solvent was evaporated and the remaining solid material taken up in 5 ml of dry THF and filtered. The filtrate was added dropwise to a stirred suspension of 0.09 g of d-biotin and 44 microliters of triethyl amine in 5 ml of dry THF. The

reaction mixture was stirred under nitrogen for one hour at room temperature. The contents were then filtered. The product biotin anhydride was isolated by removing the solvent from the filtrate.

Preparation of the chip for attachment of molecules.

To enable the attachment of molecules by vitamin B_{12} mediated carbon—carbon bond formation proximate to the surface of the electrode array chip, the chip was coated/modified with an overlaying membrane of a polysaccharide-based material. Specifically, a polygalactoside that was modified with acryloyl groups was used as overlaying membrane materials in this example. The membrane was applied by spin coating onto the chip.

Electrochemically mediated formation of carbon—carbon bonds between biotin and activated olefin groups.

A DMF solution that was 0.01M in biotin anhydride, 0.37 mM in vitamin B_{12}, and 0.032M in tetrabutylammonium nitrate was prepared. A chip was immersed in the solution and a potential difference of 3.0 V was applied between the anode and the cathode for 5 minutes. This was repeated with different pairs of electrodes as needed. After the electrochemistry was completed, chips were removed from solution and washed with deionized water and acetone.

Assay with fluorescent dye labeled molecules

The electrochemically modified chip was immersed in an aqueous solution of fluorescent dye (Texas Red) labeled streptavidin molecules having a concentration of 50 micrograms per milliliter and allowed to remain in this solution for one hour. Fluorescent dye labeled streptavidin was obtained from Vector Laboratories (Burlingame, Calif.). During this immersion, biotin molecules attached to the membrane formed a complex with the fluorescent dye labeled streptavidin molecules.

The chip was then washed with an aqueous 0.1M sodium phosphate buffer that was adjusted to pH 8.0 to remove dye labeled streptavidin that was not complexed with membrane bound biotin. The chip was now ready for evaluation by epifluorescent microscopy.

Results

Formation of carbon—carbon bond between acryloyl groups and biotin

A potential difference of 3.0 V was applied between hardwired electrodes T_1 and T_2 for 5 minutes. T_1 was the cathode and T_2 was the anode. Then, a 3.0 V potential difference was applied across hardwired electrodes T_3 and T_4. T_4 was the cathode and T_2 was the anode. Electrochemical reduction of vitamin B_{12} occurred at the cathodes. The chip was then exposed to Texas Red labeled streptavidin as described and the photomicrograph of FIG. 27 was obtained. Bright spots at the cathodes indicate the presence of biotin bound to the overlaying membrane. Control experiments were performed to rule out the possibility of unanticipated artifacts causing a false positive.

Control Experiments

To demonstrate that the observed results were due to the formation of carbon—carbon bonds between biotin and an immobilized activated olefin the following control experiments were performed. The conditions used in the control experiments were identical to the conditions used for the carbon—carbon bond forming experiments.

A first control experiment was designed to confirm that the vitamin B_{12} catalyst was necessary for the results obtained in FIG. 27. Chip was prepared with an acryloyl modified polysaccharide membrane as described. This chip was then immersed in a DMF solution identical to the DMF solution used to form carbon—carbon bonds, except that there was no vitamin B_12 added to the solution. A voltage difference of 3.0 volts between the anode and the cathode was set for 5 minutes. No observable current passed between the electrodes. At this potential difference, there are no electroactive species in solution without vitamin B_{12}. The first control chip was then exposed to Texas Red labeled streptavidin and washed in a procedure identical to the one outlined above. The results are shown in FIG. 28. No evidence of carbon—carbon bond formation is seen.

A second control experiment was designed to confirm that the biotin anhydride substrate was necessary to obtain the results of FIG. 27. A chip was prepared with an acryloyl modified polysaccharide membrane as described. The chip was then immersed in a DMF solution identical to the DMF solution used to form carbon—carbon bonds, except that there was no biotin anhydride added to the solution. A voltage difference of 3.0 volts between the anode and the cathode was set for 5 minutes. The second control chip was then exposed to Texas Red labeled streptavidin and washed in a procedure identical to the one outlined above. The results are shown in FIG. 29. No evidence of carbon—carbon bond formation is seen.

A third control experiment was designed to confirm that the activated olefin was necessary to obtain the results of FIG. 27. A chip was prepared with an unmodified polysaccharide membrane as described. The chip was then immersed in a DMF solution identical to the DMF solution used to form carbon—carbon bonds, except that there was no activated olefin attached to the overlaying membrane. A voltage difference of 3.0 volts between the anode and the cathode was set for 5 minutes. The third control chip was then exposed to Texas Red labeled streptavidin and washed in a procedure identical to the one outlined above. The results are shown in FIG. 30.

Example 6

Background

A major obstacle to exposing semiconductor devices to environments that contain ions is that the ions diffuse into the device. In particular, ions diffuse into regions of the device that have been doped with ions in a precise manner to impart particular electrical properties to these regions. An important example is the gate of a metal oxide semiconductor (MOS) transistor circuit element. Here either positive or negative ions (e.g., p-doped or n-doped) have been diffused into the gate region to make the region semiconducting. The threshold voltage and current-voltage characteristics of the transistor gate depend in a sensitive way on doping levels.

The long term reliability of many semiconductor devices depends on isolating them effectively from ionic contamination. The adhesives and encapsulants used in the semiconductor industry are treated to render the ion concentrations in these materials as low as possible, often less than parts per million.

Likewise, ion contamination represents a potential problem for utilizing devices comprising selected electrode(s) whose electrical activity is controlled by computer generated signalling because such devices would be immersed and operating in high concentration ionic solutions for extended periods of time. As a result, it is desirable to incorporate structures into such that are designed both to monitor and to obviate ion contamination.

Such devices are designed to work by scavenging ions that diffuse into the device from the solutions to which they are exposed. These contaminating ions can be scavenged

passively by reacting chemically with a material that is placed between them and the active circuitry. Alternatively, they can be scavenged actively by applying a voltage to an electrode that sets up an electric field that causes ions to migrate to the electrode and away from the active circuitry. We call these electrodes 'gettering' structures. Ion contamination can be monitored by placing transistor gates adjacent to the gettering electrodes and monitoring shifts in threshold voltage.

The time course of ionic contamination was monitored in such devices. To do this, a transistor using a ring getter electrode as a gate electrode was prepared. The MOS gate of this transistor was close to the electrochemical electrode. The MOS gate in this case is n-doped. The device was designed to allow getter voltages of up to 50 volts. The transistor design is illustrated in FIG. 31.

Results

Initial evaluation of the gettering device confirmed that there was ionic contamination at the monitoring transistor when the chips were dipped into aqueous salt solutions. FIG. 31 illustrates the shift in the threshold voltage of the transistor monitoring device after a 20 minute exposure to a 0.1 M $NaPO_4$ solution. The measured data is fit to a subthreshold MOS curve of the form $V=V_0 ln(I/Io)$.

Ions appear to contaminate the MOS gate of the monitoring transistor fairly quickly. The threshold voltage goes up, indicating that sodium ions are the primary contaminating species. The threshold voltage goes up because the conductivity of the MOS gate goes down. Since the MOS gate is n-doped, this means that positive sodium ions are, to some extent, neutralizing the negative ions that were used to dope the gate.

There is a secondary mode of contamination. It is possible that the surface layers of the chip are contaminated quickly, but the effects are not seen in the circuitry until much later. Because ions diffuse so slowly in the dielectric material of the chip, it may take some time before the ions in the surface contamination layer diffuse into the circuitry of the device. This was tested by immersing a chip for 20 minutes in an aqueous 0.1 M sodium phosphate solution. The chip was then removed from the solution, washed and dried. A getter electrode was set to 32 volts and the current monitored over time. The chip was not exposed to any solution after the initial 20 minute exposure. The results are shown in FIG. 33. The getter current continues to rise for several hours after the initial exposure to ions indicating a concomitant lowering in the threshold voltage of the gate. In other words, sodium ions are diffusing away from the gate region. The time course of the current rise follows an approximately logarithmic course for the first few hours. This is consistent with a self-screening diffusion process. Long term contamination is a problematic issue. Even though chips may seem fine after an initial exposure to salt solutions, they may fail later due to incipient surface contamination problems. Using a "getter" structure in accordance with the present invention effectively decreases such contamination.

What is claimed is:

1. A method for electrochemical placement of a material at a specific location on a substrate, which comprises the steps of:

providing a substrate having at its surface at least one electrode that is proximate to at least one molecule bearing at least one protected chemical functional group,

placing a buffering or scavenging solution in contact with the electrode at the surface of the substrate to prevent the electrochemically generated reagents from leaving the locality of the electrode,

applying a potential to the electrode sufficient to generate electrochemical reagents capable of deprotecting at least one of the protected chemical functional groups, and

bonding the deprotected chemical functional group with a monomer or a pre-formed molecule.

2. A method according to claim 1, wherein said buffering solution is selected from acetate buffers, borate buffers, carbonate buffers, citrate buffers, glycine buffers, HEPES buffers, MOPS buffers, phosphate buffers, TRIS buffers and KI solutions.

3. A method according to claim 1, wherein said buffering solution is present in a concentration of at least 0.01 mM.

4. A method according to claim 1, wherein the concentration of the buffering solution ranges from 0.1 to 100 mM.

5. A method according to claim 1, wherein said monomer or preformed molecule has at least one other protected chemical functional group at a site different from where bonding with the deprotected chemical functional group of the molecule occurs.

6. A method according to claim 1, wherein said monomer is an amino acid.

7. A method according to claim 1, wherein said preformed molecule is selected from proteins, nucleic acids, polysaccharides, and porphyrins.

8. A method according to claim 7, wherein said preformed molecule is a nucleic acid.

9. A method according to claim 1, wherein said molecule is a linker molecule or a monomer.

10. A method according to claim 1, wherein said molecule is directly attached to the surface of said substrate, is attached to the surface of said substrate via a linker molecule, or is attached to a layer of material overlaying said substrate.

11. A method according to claim 1, wherein said protected chemical functional groups are protected with an acid or base labile protecting group.

12. A method according to claim 1, wherein said at least one electrode comprises an array of electrodes.

13. A method according to claim 12, wherein said array of electrodes comprises at least 100 electrodes.

14. A method according to claim 5, further comprising sequentially deprotecting the other protected chemical functional group of the monomer or pre- formed molecule and bonding to the deprotected monomer or pre-formed molecule another monomer or pre-formed molecule.

15. A method for electrochemical synthesis of an array of separately formed polymers on a substrate, which comprises the steps of:

placing a buffering or scavenging solution in contact with an array of electrodes that is proximate to a substrate surface, said surface being proximate to one or more molecules bearing at least one protected chemical functional group attached thereto,

selectively deprotecting at least one protected chemical functional group on at least one of said molecules;

bonding a first monomer having at least one protected chemical functional group to one or more deprotected chemical functional groups of said molecule;

selectively deprotecting a chemical functional group on the bonded molecule or another of said molecules bearing at least one protected chemical functional group;

bonding a second monomer having at least one protected chemical functional group to a deprotected chemical

placing a buffering or scavenging solution in contact with the electrode at the surface of the substrate to prevent the electrochemically generated reagents from leaving the locality of the electrode,

applying a potential to the electrode sufficient to generate electrochemical reagents capable of reacting to the at least one molecule proximate to the electrode, and

producing a chemical reaction thereby.

46. A method according to claim 45, wherein said buffering solution is selected from acetate buffers, borate buffers, carbonate buffers, citrate buffers, glycine buffers, HEPES buffers, MOPS buffers, phosphate buffers, TRIS buffers and KI solutions.

47. A method according to claim 45, wherein said buffering solution is present in a concentration of at least 0.01 mM.

48. A method according to claim 45, wherein the concentration of the buffering solution ranges from 0.1 to 100 mM.

* * * * *

NANOGEN, INC

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DONALD D. MONTGOMERY, et al.

SEALED DOCUMENT

EXHIBIT C

EMPLOYEE HANDBOOK

1994

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A MESSAGE FROM NANOGEN

The ability to operate Nanogen in a successful manner is directly dependent on its employees, their quality and their spirit and how all employees work together. When Nanogen employed you, it hired the best candidate for the job. Nanogen is proud of its people and strives to operate so that all employees will have reason to be proud of Nanogen.

One of Nanogen's goals is to operate efficiently and profitably and, in turn, provide additional opportunities for all employees. The Company intends to provide meaningful jobs, career development opportunities and competitive wages and benefits.

A company operates successfully only to the degree its employees recognize and accept their responsibilities. Each employee must do his or her part to maintain a working climate that will lead to overall success. To do this, work must be performed conscientiously with full use of skills and abilities in a safe and efficient manner.

This handbook has been prepared for the information and guidance of everyone working at Nanogen. These guidelines are intended to cover the procedures and policies that most often apply to day-to-day activities. Some of the information may change from time to time since the policies are under constant review. You may also have specific policies which relate to your individual department. It is part of your responsibility to know and support those policies, as well as those provided in this handbook.

Nanogen hopes this booklet will answer most of your questions. If, however, you have additional questions or suggestions, please feel free to speak to your supervisor or the Human Resources Manager. They are here to assist you and encourage an open-door policy among all at Nanogen.

On behalf of Nanogen, welcome to our team!

Howard C. Birndorf
Chairman & Chief Executive Officer

and

Tina S. Nova, Ph.D.
President & Chief Operating Officer

EQUAL OPPORTUNITY EMPLOYMENT

The purpose of this policy is to delineate the areas encompassed by equal employment opportunity and to define the parties responsible for compliance.

It is the intent and resolve of Nanogen (the "Company") to comply with the requirements and spirit of the law in the implementation of all facets of equal opportunity. Our continued growth and success is dependent on the full use of all employee resources. Nondiscriminatory employment decisions will continue to be the policy and practice of Nanogen.

It is the policy of Nanogen to provide equal employment opportunity to all qualified people, regardless of race, color, religion, sex, national origin, age, handicap or veteran status. This policy encompasses all employment practices including, but not limited to the following:

1. Hiring, placement, supervision, promotion/upgrading, transfer or demotion.
2. Recruitment, advertising or solicitation for employment.
3. Treatment during employment.
4. Business travel.
5. Rates of pay or other forms of compensation.
6. Selection for training.
7. Layoff, recall from layoff or termination.
8. Benefits, education tuition reimbursement and social and recreational programs.

Responsibility for Compliance

It is the responsibility of each manager to see that the Company policy of equal opportunity is communicated and understood by all employees within his or her organization. Each manager must also ensure that within his or her organization, applicants and employees are treated equally with respect to their abilities and without regard to their race, color, religion, sex, age, national origin, handicap or veteran status.

It is the responsibility of each manager to ensure that compliance with the equal employment opportunity policy is a part of the overall operating plan.

It is the responsibility of the Company management to analyze and assess personnel actions to ensure equal employment opportunity.

Nanogen is proud to be an equal opportunity employer.

CONDITIONS OF EMPLOYMENT

Upon acceptance of employment with Nanogen, all new employees must agree to certain conditions of employment. These conditions include, but are not limited to, the following:

1. Completion of an Application for Employment;
2. Submission of proof to legally work in the U.S.;
3. Execution of the Blood Draw Consent Form;
4. Completion of the Proprietary Information and Inventions Agreement; and
5. Written acceptance of this Handbook, the Biosafety Handbook, Radiation Safety Handbook, Chemical Safety Handbook and the Injury and Illness Prevention Program Handbook.

A WORD ABOUT UNIONS

We at Nanogen are sincerely interested in the personal development and happiness of each of our employees. We believe that Nanogen can best promote the achievement of personal and company goals through direct and open communication. It is our policy to deal with our employees fairly and honestly and to respect and recognize each as an individual.

We firmly believe that the best interests of our employees can be served without third-party interference. We greatly value our ability to treat our employees as individuals without subjecting them to burdensome costs, complicated rules and costly work stoppages. Therefore, we do not believe that unionization is necessary or desirable. If you ever have questions on this subject, feel free to discuss them with your supervisor.

OUR RELATIONSHIP

While we hope our relationship will be long and mutually beneficial, it should be recognized that employment with Nanogen is at the will of either the employee or employer. This means that the employee may resign at any time and similarly, Nanogen is free to conclude an employment relationship at any time with or without notice. There is no promise that employment will continue for a set period of time, nor is there any promise that your employment will be terminated only under particular circumstances. No one except the Chairman of the Company and the Company President has the authority to make representations inconsistent with this policy.

CODE OF EMPLOYER-EMPLOYEE RELATIONS AND STANDARDS OF CONDUCT

It is the policy of the Company to announce to all employees the fundamental principles and mutual rights and obligations comprising the relationship of employment between the Company and its personnel.

A. In its continuing effort to implement fair and effective Personnel Policies and Practices, the Company pledges:

1. To employ people on the basis of their qualifications and with assurance of equal opportunity and treatment regardless of race, religion, color, sex, age, national origin, handicapped or veteran status;
2. To provide salaries and other employee benefits which bear a fair and reasonable relationship to the work performed;
3. To establish reasonable hours of work;
4. To maintain safe and healthful working conditions;
5. To place employees in the kind of work best suited to their abilities;
6. To provide training to those whose needs, capabilities and desires warrant such training in relation to their responsibilities at Nanogen;
7. To welcome constructive suggestions which relate to methods, procedures, working conditions and the nature of the work performed;
8. To establish procedures for employees to discuss freely any matter of interest or concern with their immediate supervisors or department managers; and
9. To permit each employee as much discretion and responsibility as is consistent with a well-coordinated and effective operation.

B. The Company expects all employees:

1. To give a productive day's work to the best of their abilities and skills;
2. To arrive at their assigned work location and begin work on time;
3. To use Company equipment and supplies in a wise and proper fashion;
4. To demonstrate a considerate, friendly and constructive attitude toward fellow employees; and
5. To adhere to all Company Rules, Regulations and Policies.

C. The Company retains the right to exercise customary managerial functions, including the following rights:

1. To supervise, assign, discipline and dismiss employees;
2. To determine and change starting times, quitting times and shifts;
3. To transfer employees within departments or into other departments and other classifications;
4. To determine and change the size of and the qualifications of the work force;
5. To establish, change and abolish its Policies, Practices, Rules and Regulations;
6. To determine and change methods by which its operations are to be carried out;
7. To assign duties to employees in accordance with the Company's needs and requirements and to carry out all ordinary administrative functions; and
8. To exercise all other rights inherent in the Company's right to manage the business.

Consistent with Nanogen's policy of keeping employees informed of what is expected, the following are examples of some, but not all, violations of this doctrine. Such violations will lead to disciplinary action, based on the circumstances of the individual case, up to and including termination.

- Disregard for established safety regulations;
- Excessive tardiness and absenteeism;
- Mishandling of Company funds;
- Destruction or misuse of Company property or supplies;
- Disorderly conduct;
- Dishonesty in any form, including falsification of records or Company documents;
- Incompetence and inefficiency;
- Interference with, or intimidation of, fellow employees;
- Disclosure of confidential company information to non-employees;
- Disclosure of any information or giving a reference to any person regarding an employee or former employee;
- Unauthorized possession or use of drugs, drug paraphernalia or intoxicants such as alcohol, etc.; and
- Carrying weapons of any kind.

We appreciate your wholehearted cooperation in the observance of Nanogen's policies, which are essential to ensure good working conditions, order and safety for all employees.

REHIRE POLICY

Nanogen will consider applications from former employees together with other qualified applicants for available positions. Nanogen will, however, take into consideration the circumstances surrounding the employee's departure.

EMPLOYEE STATUS DEFINITION

The purpose of this policy is to define part-time and temporary employee status and to describe benefits applicable to part-time and temporary employees. This policy is not intended to apply to student interns, co-op students, technical consultants or others whose employment is governed by programs or agreements established between Nanogen and an educational institution or by any other contractual agreement. Benefits for such individuals are specified in the particular agreement or contract through which they are employed.

Part-Time Employee
A part-time employee is any employee who is regularly scheduled to work fewer than 30 hours per week. Such employees may be either "temporary" or "regular" employees.

A. Temporary Employee

A temporary employee is any employee paid through the Company payroll whose employment is of a specified and limited duration usually not exceeding three months. An extension of a temporary work classification for an additional three month period, or less, may be granted if found to be necessary upon management review. Such employees may be either "part-time" or "full-time." Because of the limited duration of their employment, temporary employees are not eligible for any benefits or paid leave time.

1. This designation of "temporary" applies to all students, whether working part-time or those who work during the summer. The three-month limitation mentioned above does not apply to students.

2. People hired through temporary agencies are employees of that agency and not of Nanogen.

B. Regular Employee

A regular employee is any employee paid through the Company payroll whose employment duration is not specified. Such employees may be either "full-time" or "part-time."

1. Regular Full-Time Employee (works >30 hours per week)
   - Eligible for group insurance benefits
   - Pay for vacation, sick time, holidays, etc., based on regular wages
   - Vacation and sick leave earned as indicated for full-time employees in the Benefits section of this handbook

2. Regular Part-Time Employee (works <30 hours per week)
   - Not eligible for group insurance benefits
   - Pay for vacation, sick time, holidays, etc. prorated
   - Number of hours of vacation and sick leave prorated
   - If not working daily, holidays, bereavement leave, jury duty, etc. are paid only if they fall on a scheduled work day

HOURS OF WORK

The normal work week for full-time and hourly employees is eight hours per day, five days per week, with a minimum of a thirty-minute to a maximum of one-hour lunch period scheduled to meet the department's needs. In addition, hourly employees receive two ten-minute breaks per day. Part-time employees receive individualized schedules upon hire.

TIME SHEETS

Your time sheet is your bill to the Company for the hours you work. It must accurately reflect non-exempt employees' hours so that pay can be correctly calculated. Any time-off should be documented by all employees in order to update vacation and sick time balances. You are on the honor system--falsification of time sheets or other Company records, may be grounds for immediate termination of employment.

OVERTIME

Business conditions may, on occasion, require overtime and should be considered as part of the job. Although it is Nanogen's intent not to overuse the concept of overtime, if it is in the best interest of the Company, overtime work may be scheduled.

- Non-exempt employees are not permitted to work any overtime hours without prior authorization.

- Exempt employees are not eligible to receive overtime compensation.

- All overtime must be accurately documented on your time card so pay will be calculated accordingly.

- Time worked in excess of eight hours per workday or 40 hours per workweek is considered overtime. Overtime is compensated in the following manner:

HOURS WORKED	RATE OF OVERTIME PAY
In one day, over eight hours and up to and including 12 hours	Time and one-half
In one day, over 12 hours	Double time
In one week, over 40 hours	Time and one-half
Seventh consecutive workday up to and including eight hours	Time and one-half
Sixth and seventh consecutive workday: any work over eight hours	Double time

Sick days, holidays, vacation time, etc., do not constitute time worked for purposes of calculating overtime. Only hours actually worked are counted toward overtime calculations.

PAYDAY

You will receive your paycheck by 5:00 p.m. on the 15th and the last day of each month. (There may be a delay with automatic deposit.) The checks will be distributed one day earlier should those dates fall on a holiday, or on Friday should they fall on a weekend.

Standard deductions will be made from your gross pay for Federal and State Income Taxes, Social Security (FICA) and State Disability, in accordance with government requirements.

If you have any questions about your paycheck or your deductions, do not hesitate to speak with the Finance Department.

PERFORMANCE REVIEWS/WAGE ADJUSTMENTS

It is Nanogen's philosophy that every employee will have an opportunity at least once a year to have a formal, two-way discussion regarding job performance and career development. Although informal coaching/counseling sessions should take place on a frequent and routine basis, a separate in-depth evaluation of the individual's job performance, career goals and developmental needs will be conducted after every twelve months of employment with the Company. Merit adjustments to your wages may occur at this times.

Performance is a factor in all reviews, however, wage adjustments are not automatic. We believe in compensating employees based on merit, quantity and quality of production, professional attitude, ability and initiative. Those employees who demonstrate outstanding performance will be compensated accordingly.

TERMINATION OF EMPLOYMENT

Should you wish to terminate your employment with Nanogen, a two week written notice is preferred. This notice should be submitted to your supervisor. If asked for a reference on a former employee, the Human Resources Representative shall verify only the date of your employment and last position held. No other information will be released.

In order for Nanogen to conduct its business efficiently and to meet its goal of providing a quality working environment, all employees are expected to meet the standards of conduct and policies set forth in this handbook. In most cases, if you have a performance problem, your supervisor will give you corrective action warnings and an opportunity to improve. These corrective action warnings may include informal verbal warnings as well as written Counseling Statements which will kept on file in your personnel record. Failure to improve performance will result in disciplinary action up to and including termination.

CONFLICTS OF INTEREST

In order to promote a high standard of conduct in the performance of your job, it is important that you avoid conflicts of interest. A conflict of interest is a situation where your private interests or outside economic interests actually or potentially interfere or conflict with the duties and responsibilities with Nanogen. A number of factors, including your position and responsibilities, may determine whether a potential conflict exists. If you need advice as to whether a conflict of interest may exist, please consult your supervisor.

To aid in avoiding conflicts of interest, Nanogen requires that you do not accept outside employment or engage in conflicting business activities without prior approval of the Company President. Nanogen recognizes the right of its employees to spend their non-working time away from the job as they please. It does, however, require that activities away from the job follow the guidelines below:

- They must not adversely affect the employee's job performance or compromise the Company's interest;

- Proprietary Company information, including trade secrets, which could benefit other firms and individuals, must not be transmitted outside of the Company without prior written authorization from the Company President;

- Use of "inside" information about the Company by any employee for personal profit;

- Avoidance of a loss in trading in securities of the Company before the information has been fully released and is available to the public is forbidden; and

- Relatives or co-habitants of current employees may not be hired if such hiring would create a supervisory relationship with that relative or would put the employee in a position to influence that relative's employment, salary or other related management considerations.

For these purposes, "relative" means spouse, mother, father, children, sisters, brothers, mothers- and fathers-in-law, sons- and daughters-in-law, cousins, aunts and uncles.

PAID HOLIDAYS

Since the dates of certain holidays vary each year, a holiday schedule will be distributed every January to each employee. If you have not received a schedule for this year, you may obtain one from the Human Resources Department.

Eligibility

1. All regular, full-time employees are normally eligible for paid holidays.

2. For regular, part-time employees, holiday pay will be prorated. If an employee is normally scheduled to work less than every day, a holiday will only be paid if it falls on a normally scheduled work day.

3. Temporary employees are not eligible for paid holidays, however, if a temporary employee is required to work on a paid holiday, he or she will receive double time for hours worked.

4. Employees must work the last scheduled day before a holiday and the first scheduled working day following the holiday in order to be eligible for holiday pay/holiday premium pay unless time-off on these days has been excused with pay (e.g., vacation, sick leave). The immediate supervisor has the option of recommending that an employee not receive holiday pay if there is a pattern of absenteeism prior to or after holidays.

Holiday Premium Pay

Scheduled work on holidays is discouraged since the purpose of holidays is seen by the Company as a provision for employee relaxation. If an employee is required to work on a scheduled holiday, that employee will be compensated as follows:

A. Regular Non-Exempt, Full-Time Employees

1. Normal pay for the number of hours the employee is normally scheduled to work, which is defined as holiday pay.

2. Double time for all hours actually worked on the holiday, which is defined as holiday premium pay.

B. Regular, Non-Exempt, Part-Time Employees

1. If a holiday falls on a part-time employee's regularly scheduled workday and the employee is required to work, the pay will be the same as above (A1 and A2). Normal pay is defined as the usual prorated pay for a part-time employee.

2. If the holiday falls on a day not regularly scheduled for the part-time employee and the employee is required to work, he or she will be paid double time for all hours actually worked.

C. Exempt Employees

Exempt employees who are asked to work on a Company paid holiday will be given another day off with pay.

VACATIONS

Since Nanogen recognizes the value of its employees' time away from work, each employee is strongly encouraged to take all vacation accrued each year. Vacations are based on length of service with Nanogen and accrue pro rata each pay period (commencing on the date of hire) as follows:

Years of Service	Amount of Vacation
Less than 1 year	10 days
1 but less than 2 years	11 days
2 but less than 3 years	12 days
3 but less than 4 years	13 days
4 but less than 5 years	14 days
5 but less than 6 years	15 days
6 but less than 7 years	16 days

7 but less than 8 years	17 days
8 but less than 9 years	18 days
9 but less than 10 years	19 days
10 years and over	20 days

Employees are encouraged to take their vacation during the year that it is earned. If this is not possible, each employee may accumulate a maximum of 150% of their annual eligible amount of vacation (1.5 x Amount of Vacation, above). Anytime an employee's maximum accrual is reached, their monthly accrual will stop and does not begin again until the first day of the month following the one in which accumulated vacation time is used.

A. Eligibility

1. All Regular, Full-Time Employees: Vacation is earned pro rata as in the above schedule.

2. Regular, Part-Time Employees: Vacation hours earned are prorated based on the average number of hours worked per week.

B. Vacation Pay

1. Salary during a vacation is at the same straight time rate normally earned by an employee. Salary for regular, part-time employees is prorated.

2. If one or more pay days fall within an employee's vacation, the employee may receive those paychecks on the last work day prior to the vacation, provided a salary advance has been requested at least one week prior to the first day of vacation. (Ask the Human Resources Department for the proper form.)

3. Terminating employees who have completed three months of continuous employment will receive pay for all unused vacation time accrued through their last day worked.

C. Restrictions

1. New employees must complete three months of continuous employment before taking vacation. Exceptions to this may be granted under special circumstances.

2. Approval for vacation must be secured from your supervisor at least two weeks prior to your first day of vacation (a Vacation Request Form must be used). Whenever possible, requests for

vacation time will be granted. However, if the vacation falls during a particularly busy time in your department, you may be asked to reschedule your vacation to a more convenient time.

3. Vacation requests which would result in an employee being in a negative accrual status after their vacation is taken must be (a) pre-approved by the employee's supervisor and Company management, (b) not be detrimental to Company staffing requirements for productivity, and (c) under no circumstances place the employee in a negative accrual status which exceeds 40 hours. Negative accruals are not available to part-time employees.

4. An employee has a liability to "pay back" any negative accrual through future accruals against the balance or deductions against their final paycheck if they leave the employ of the Company.

5. Vacation time should be used in increments of full days.

6. Vacation time does not accrue during leaves of absence or other unpaid absences from work.

LEAVES OF ABSENCE

Nanogen offers several types of leaves of absence with conditions detailed below. It should be noted that employee benefits do not accrue during a leave of absence. All benefits would resume upon your return to work.

Employees on medical or maternity leave are eligible to apply for state disability insurance administered by the State Employment Development Department. Further information can be obtained by contacting either the Nanogen Human Resources Department or any office of the Employment Development Department. Employees are encouraged to avail themselves of this benefit.

Medical Disability Leave

After six months of employment with the Company, regular full-time employees are eligible for unpaid disability leaves of absence, which will be granted for disabilities due to illness or injury. The leave of absence will begin after a period of eight consecutive days of absence (verified by a doctor's written statement) and shall be granted for a period equal to the actual duration of the disability up to a maximum of four months from the date the employee becomes disabled.

The Company will pay for the employee's group health insurance benefits during any disability leave. Employees will be required to use any accrued sick leave at the beginning of their leave. Vacation time cannot be used to extend a leave of absence, but may be taken in lieu of a leave of absence.

An employee must submit a Leave Request to his or her supervisor. The request should give maximum available notice of the anticipated beginning and ending dates of the leave. A physician's statement must also be submitted at the beginning of the leave and every 30 days thereafter stating that the employee is still disabled, and at the end of the leave stating that the employee is capable of returning to work.

The Company will seek to keep an employee's position open during a disability leave for illness or injury, but there can be no guarantee of re-employment. If the employee, as soon as he or she is capable, fails to return to his or her prior position or a comparable one, if either is available, the Company will consider the employee to have voluntarily resigned.

No employee will be entitled to take more than four months of leave during any twelve-month period.

Maternity Leave

Employees, including new hires, are eligible for unpaid maternity leave. The leave of absence will be granted for a period of the actual disability, up to a maximum of four months. Employees will be required to use any accrued sick leave at the beginning of their leave. Maternity leave cannot be extended beyond the above stated periods.

The Company will pay for the employee's group health insurance benefits during maternity leave if the employee is eligible for group health benefits prior to the leave of absence.

An employee must submit a Leave Request to her supervisor. The request should give maximum available notice of the anticipated beginning and ending dates of the leave. A physician's statement must also be submitted at the beginning of the leave and every 30 days thereafter stating that the employee is still disabled, and at the end of the leave stating that the employee is capable of returning to work.

Upon the employee's return from maternity leave, she will be returned to her original job, unless the job ceases to exist because of business necessity or if the Company cannot preserve the job and operate safely or efficiently. In such a case, the Company will seek to provide the employee with a substantially similar position. If the employee, as soon as she is capable, fails to return to her prior position or a comparable one, if either is available, the Company will consider the employee to have voluntarily resigned. If the employee's prior position or a comparable one is not available at the end of the leave, the employee will be given preference for the next available comparable position for which the employee is qualified.

Military Leave
If you enter military service and are required to attend yearly Reserves or National Guard duty, you may apply for an unpaid, temporary military leave of absence.

Employees are required to provide a copy of military orders to their supervisor at least two weeks prior to the time that absence from work is required.

Emergency/Personal Leave
Requests for emergency/personal leave without pay will be considered on an individual basis. Such requests should be submitted to your supervisor.

Bereavement Leave
All regular employees may take up to three paid days off for bereavement leave provided the deceased was related to the employee by being a spouse, child, parent, sister or brother, grandchild, grandparent, mother-, father-, sister- or brother-in law, or other relative who was a resident in the employee's household at the time of death.

Jury Duty
If you are called to perform your civic duty by serving on a jury panel, you must present a copy of your summons to your supervisor as soon as you receive it. This will give your supervisor time to find a suitable replacement for the time you are away. If you are called as a juror during a particularly busy time in your Department, we may ask you to request the court to postpone your jury duty to a more convenient time.

An employee who has been granted a leave of absence for temporary jury duty will receive regular time compensation for time lost up to a maximum of 10 days, less the compensation received by the employee for the jury duty. Paid time off from work covers only the time you are actually required to be at the courthouse. If you are on telephone standby, you are expected to report to work and perform your job while waiting to be called to serve on a jury.

After completion of jury duty, the employee must provide the supervisor with all compensation records received.

EDUCATIONAL ASSISTANCE

Nanogen's Educational Assistance Program is designed to encourage eligible employees to continue self-development in their current jobs, or other jobs considered to be in the Company's interest, by providing financial assistance for educational programs directed toward this objective.

A. Eligibility

Regular, full-time employees are eligible for the Educational Assistance Program. Course approval is required from the employee's supervisor, department Director and the Company President, prior to registration.

B. Reimbursement

Nanogen will reimburse eligible employees the cost of tuition, books and laboratory fees up to a maximum of $500 per calendar year. The following course grades must be received for this reimbursement:

Undergraduate Courses - a minimum of a grade "C" or its equivalent.
Graduate Courses - a minimum of a grade "B" or its equivalent

C. Courses

Courses must be taken outside of regularly-scheduled working hours. Exceptions require the approval of the supervisor, department Director and Company President. Courses must be taken at a Company approved and accredited school, college or university. Reimbursement will be made upon submission of original receipts and proof of course grades. Meals, transportation and parking are not reimbursable.

D. Seminars and Workshops

Attendance and reimbursement for professional seminars and workshops are at the discretion of the appropriate department manager and are not included in this program.

OTHER BENEFITS

From time to time the Company may provide additional benefits to its employees. These may be scheduled on a one-time only or a continuing basis. Some examples could include the following:

- "TGIF" -- An extra "thank you" for a job well done at the end of the week.
- Winter Holiday Get-Together -- To show appreciation for your efforts throughout the year and to celebrate the holiday season.

WORK GUIDELINES AND POLICIES

SAFETY

We are concerned about your safety and that of those with whom you work. For this purpose, a Biosafety Handbook, Radiation Safety Handbook, Laboratory and Chemical Safety Handbook and an Injury/Illness Prevention Handbook have been developed.

The Company will continue to provide a clean, healthy and safe place to work and will conduct safety meetings periodically. With an alert and safety attitude, you can help eliminate painful and costly accidents. You can help by consistently following the guidelines below:

- Report hazards or unsafe conditions to your supervisor immediately;
- Wear proper clothing that will not interfere with the performance of your job;
- Report all injuries, regardless if they appear minor, to your supervisor immediately. In the event of a life threatening injury to another employee, immediately contact public emergency assistance for direction or assistance;
- Never perform a job that you feel is unsafe; and
- Think about safety as you plan and perform your work.

Nanogen is committed to providing a safe environment to its employees and your adherence to our safety rules and procedures is mandatory and will be considered in your performance reviews. Continued and/or willful violation of our safety procedures will result in disciplinary action up to and including termination.

SUBSTANCE ABUSE

The use, possession or sale of alcohol or non-prescription, illegal drugs by employees is strictly prohibited while on duty or on Company premises. Reporting to work under the influence of alcohol or non-prescription, illegal drugs is also strictly prohibited. Nanogen reserves the right to require employees to agree to inspection of their persons and/or personal property. Withholding consent to such an inspection, or any violation of this policy, will result in disciplinary action up to and including termination.

SOLICITATION AND DISTRIBUTION

All employee solicitations for gifts, birthdays, cosmetics, cookies, and all other purposes must be conducted during non-working time and out of the work area. Solicitation of one employee by another is prohibited while either person is on working time. Working time is all time when an employee's duties require that he or she be engaged in work tasks, but does not include an employee's own time, such as lunch period and scheduled breaks.

SEXUAL HARASSMENT

It is the policy of Nanogen to provide employees with a work environment free from all forms of discrimination, including sexual harassment. This policy is an adjunct to the Company's Equal Employment Opportunity Policy.

A. Definition

Sexual conduct where submission to or rejection of such conduct affects terms or conditions of employment; that substantially interferes with an employee's ability to perform the job or that creates a hostile work environment.

According to the EEOC, Title VII of the Civil Rights Act of 1964 does not limit all conduct of a sexual nature in the workplace, but rather only unwelcome sexual conduct that is a term or condition of employment. "Unwelcome sexual conduct" constitutes sexual harassment when submission to sexual conduct is made either explicitly or implicitly a term or condition of an individual's employment.

Sexual harassment includes, but is not limited to, the following:

- Making unwelcomed sexual advances or requests for sexual favors or other verbal or physical conduct of a sexual nature an implicit or explicit condition of an employee's continued employment;
- Making submission to or rejections of such conduct the basis for employment decisions affecting the employee;
- Unreasonably interfering with an individual's work performance by such conduct; and/or
- Creating an intimidating, hostile or offensive working environment by such conduct.

B. Problem-Solving Procedure

Any employee who believes he or she has been the subject of sexual harassment should report the incident immediately. At the discretion of the employee, the report may be made to either the Human Resources Director or to the Company President. Allegations of sexual harassment will be investigated immediately and without embarrassment or negative reaction to the reporting employee.

Treating people differently on the basis of their sex is breaking the law, and if sexual harassment has occurred, adequate steps will be taken to eliminate the illegal behavior. The Company will take swift and appropriate corrective action, up to or including termination, if after the investigation an employee is found to have sexually harassed another employee.

EMPLOYEE BENEFITS

Nanogen recognizes that its success depends upon your contributions. That is one reason why we are committed to a fair and competitive compensation program. With the high cost of health care, a very important part of your total compensation is Nanogen's comprehensive employee benefits - your "hidden paycheck."

Nanogen pays 100% of the cost of group medical, dental and life insurance for its eligible employees. If you elect coverage when you are hired, your insurance will be effective no later than the first of the month following your date of hire. The Company will also pay 70% of medical and dental insurance costs for dependents, with the employee responsible for the remaining 30% of the cost for dependents. A 125 Flex Plan is available to all employees as well. Detailed information about insurance benefits is contained in a booklet which will be given to you when coverage is elected.

WORKERS' COMPENSATION

On-the-job injuries are covered by our Workers' Compensation Insurance, which is fully paid for by the Company. If you are injured on the job, no matter how minor it may seem, you must report the incident immediately to your supervisor or the Human Resources Representative.

OTHER INSURANCE

The Company will pay its portion of the following:

FICA -- Federal Insurance Contribution Act (Social Security)
FUTA -- Federal Unemployment Insurance
SUI -- State Unemployment Insurance

In accordance with state law, withholdings are also made for State Disability Insurance, a wholly employee-supported fund.

PAID SICK LEAVE

Since the progress and success of Nanogen is dependent upon the productivity of all our employees, good attendance is encouraged and expected. However, it is in the best interest of an employee who is ill or injured not to report to work.

A. Eligibility

Regular, Full-Time. Employees: Eligible for up to 48 hours of sick leave per calendar year. New full-time employees are eligible for a prorated number of sick days based on the number of days employed during the year.

Regular, Part-Time. Employees: Number of hours of sick leave prorated based on average number of hours worked per week, also prorated as for above new employees.

Temporary Employees: Not eligible for paid sick leave.

B. Rate of Pay

Sick pay will be paid at the same straight time rate normally earned by the employee (regular wages). For part-time employees, pay for each sick day will be prorated by multiplying the employee's normal pay rate times the number of hours worked per day in his or her normal part-time schedule.

Absences of less than one full work day will be charged at hour intervals.

C. Restrictions

Sick leave will not accumulate from one calendar year to the next. Unused sick leave will expire on December 31, except in instances where an employee's illness extends into the next calendar year. When this occurs, sick leave for the year in which the illness occurred will be provided, up to the length of the illness or the amount of the employee's remaining sick leave, whichever is less.

Should an employee be on sick leave or unpaid medical leave of absence on December 31, sick leave benefits will not resume until the employee has returned to work for at least 31 days.

The immediate supervisor has the option of recommending an employee not receive sick pay if there is a pattern of absenteeism or other abuse of leave time.

In order to be eligible for paid sick leave, an employee must personally notify his or her supervisor before the beginning of each work day.

If an employee is absent the day preceding or following a holiday or weekend, the Company reserves the right to require a written statement from the attending physician.

Sick leave is not earned by an employee during a leave of absence.

If you are sick more than six days consecutively, a note from your physician will be required before you may return to work.

You may use up to 24 hours of your sick leave annually to attend to the illness of a member of your immediate family. The term "immediate family" refers to an employee's father, mother, spouse and children. Time off in excess of the 24 hours must be taken as vacation time.

COMMUNICATION

As the Company grows, our success will depend in large part on our ability to communicate our ideas with one another in a timely and accurate fashion. Regular, active and honest communications will help us share our thoughts and successfully develop individually and as a team.

Open Door Policy: When you have a question about your job or employment policies, you are encouraged to speak with your supervisor. If you wish to discuss the matter further, we encourage discussing it with the Human Resources Manager or the Company President. When a problem exists and you need help, "let's talk about it" is the best policy.

Bulletin Board: Information of interest and importance to you as a Company employee will be regularly posted on a Company bulletin board.

Problem Solving: We hope that your employment with us will be problem-free. Nevertheless, we know that misunderstandings and problems occur. When misunderstandings or problems arise, we encourage you to bring the matter to our attention as soon as possible. Nearly all problems and questions can be resolved simply and fairly, or avoided entirely, if we learn of them promptly.

If you have a problem or complaint, there are steps you can take to get it resolved:

1. Discuss your problem with your immediate supervisor. Your supervisor knows your position and your job. He or she is in the best position to help because he or she works with you and is interested in seeing that you are treated properly.

   If your problem involves your supervisor or you do not wish to discuss the matter with him or her, you may contact your department manager. Also, feel free to call upon the Human Resources Representative.

2. If you are not satisfied after speaking with your supervisor, you should talk to your department manager.

3. If, at this point, you still wish additional input, the Company President can then be called upon.

Keep in mind that prompt discussion of a problem usually works best. With the passage of time, you, or the other persons involved, may forget the facts or confuse the issues that led to the problem. If you want to get the fairest, most accurate solution to your problem, discuss it when it happens.

Your input in the communication process will be greatly appreciated.

PERSONAL TELEPHONE USE

Personal calls, both incoming and outgoing, are discouraged. However, the Company recognizes that there may be times when personal calls must be made or received during business hours. Such calls should be held to a minimum and must not interfere with your work. You are encouraged to make personal calls during lunchtime. When you must make a personal long distance call, the call must be billed to your home phone number.

BUILDING SECURITY

Employees must use their card key or keys to enter the Company, before and after business hours. All interior doors between laboratory and non-laboratory areas are to remain closed when not in use.

The last employee to leave each main area of the Company each evening should check to make sure that all doors in that section are locked, the lights turned off and designated equipment turned off. Laboratory equipment not in use should be turned off by the last technician to leave the lab. Employees should lock their offices and file drawers each evening.

The Company cannot assume liability for loss of personal property due to theft or carelessness while on Company premises. You are urged to exercise routine precautions to protect your personal property. If you do not wish the janitorial crew to enter your office, place your wastebasket outside your door, close and/or lock your office door.

VISITORS

When all visitors come to the Company, they will be greeted by the Receptionist, sign in, and be given a Visitor's badge. Visitors shall be allowed in work areas only under the supervision of a supervisor or manager.

1. Escorted visitors must be escorted by an employee at all times. They are not authorized to be in the facility without an escort. Visiting sales representatives are always escorted visitors. (All visitors are presumed to require an escort unless there is a specific reason for an exception as defined in #2 below.)

2. Unescorted visitors will be limited to repair people here for an extended period of time or affiliates of the Company.

3. After hours' visitors includes all visitors on the premises outside the standard business hours. After-hours' visitors are the sole responsibility of the employee who invited them.

4. All visitors must sign out when leaving the Company.

5. Neither cameras nor recording devices are allowed to be used by visitors in the Company facilities without the express permission of the Company President.

PURCHASING PROCEDURES

The following general rules apply to all purchases:

1. All purchases must be ordered by the authorized Nanogen Purchasing Agent.

2. All purchases of supplies, materials, equipment or services require a previously approved purchase order requisition, check request or petty cash request. Purchases or commitments made without proper approval will not be honored by Nanogen until the proper documentation is prepared and approved.

3. All purchase order requisitions and check requests will require "one over one" approval (i.e., the requisitioner's supervisor, at a minimum,

must approve the requisition or request) regardless of the dollar amount.

4. Purchases of radioactive material will need the approval of the Radiation Safety Officer before the order is placed.

5. Purchases of biohazardous material will need the approval of the Radiation Safety Officer before the order is placed.

6. Purchases of computer hardware or software will need the approval of the Company President before the order is placed.

7. Purchases or rentals of office or laboratory furniture and equipment should be coordinated through the Purchasing Department.

The general use of each form is outlined below (see Appendix for samples).

A. Purchase Requisition/Order Form

A Purchase Requisition is used for all purchases, except petty cash purchases.

The purchase order requisition describes the full purchase terms and conditions.

The dated signature of all appropriate approval levels as defined below:

Total $ Amount	**Signatures Required**
Up to $250.00	Supervisors
Up to $500.00	Managers and Scientists
Up to $1,000.00	Directors
$1,000.00 - $5,000.00	Vice Presidents
Over $5,000.00	Company President

After the requisition has been completed, it should be forwarded to Purchasing. Once the requisition has been reviewed by Purchasing, the proper signatures will be obtained and a Purchase Order Number is assigned and the order is placed with the vendor. The pink copy of the order is returned to Receiving as notification of the item expected to arrive and the expected date of arrival.

B. Check Request Form

Generally, a check request should be used for purchases of materials or services for which the vendor requires prepayment or payment

immediately upon delivery (i.e., subscriptions, seminars, postage, C.O.D. shipments, etc.) in addition to the regular purchase order requisition and procedures. The exact dollar amount, including any taxes or delivery fees, must be specified.

All items on the form must be completed by the requestor. The description section of the request should contain as much information as possible to fully describe the materials or services to be purchased. This could include quantities, catalog numbers, descriptions, delivery terms, price per item, etc.

Have the request approved by your direct supervisor and submit it to the Purchasing Department no later than five days prior to the due date. Higher level managers as dictated by the total amount of the check and the table listed under purchase requisition will be obtained by the Purchasing Department.

The check will be issued by Accounting and mailed to the payee.

The requestor is responsible for the procurement of the materials or services. After the purchase is completed, supply the Purchasing Department with receipts or other back-up.

C. Petty Cash Request Form

The Petty Cash Request is used for the following: (a) cash expenditures under $20.00, (b) expenditures of small amounts, when the exact amount is not known prior to the purchase, and (c) all "cash required" purchases.

An approved Petty Cash Request is required prior to the issuance of cash to the requestor.

After the petty cash purchase is made, a receipt is required for all petty cash transactions.

Be as descriptive as possible with the quantities, catalog numbers, descriptions, and price estimates if the exact amount is not known.

The requestor is responsible for the procurement of the materials or services. After the purchase is made, return the change and a receipt to Accounting.

D. Purchase Order Procedure

1. Fill out a Purchase Order Requisition with the preferred vendor, requested date of delivery, catalog number and price.

2. Obtain supervisor's signature.

3. Submit to Purchasing Department.

All forms mentioned above can be obtained through the Purchasing Department.

COMPUTER HARDWARE AND SOFTWARE

Computers in the laboratories are for the shared use of employees, so it is important that basic etiquette rules are observed. Any questions regarding computer hardware and/or software can be directed to the Computer Systems Administrator.

1. Computer use should be restricted to company business and access time shared on basis of priority.

2. Applications should be closed and "trash" emptied at the conclusion of the individual's session on the computer.

Most of the computers at Nanogen will be interconnected through an Ethernet network. This means that many of the functions performed on a single computer can affect all of the others. For this reason and as good computer operational practice, Nanogen requires adherence to the following guidelines:

1. All disks inserted into the computer should be virus-screened prior to use.

2. All software loaded onto computers shall be pre-approved by the Company President, obtained by Nanogen, and licensed to the Company.

3. Computer data stored on the server will be saved daily on a tape back-up system for protection against damage or loss. Individual users are responsible to save valuable company information to the server to ensure its protection.

EXPENSE REIMBURSEMENT & EXPENSE REPORTS

It is Nanogen's policy to reimburse employees for reasonable expenses incurred on behalf of the Company, within and subject to the current guidelines set by the Internal Revenue Service. Personal automobile use for company business (but not for commuting to and from work) will be reimbursed at the legal mileage rate,

(currently .28¢ per mile). At the onset of your travel planning, a Travel Request form (see Appendix) should be submitted to the Accounting Department. This form requires approval of your supervisor and should be submitted, whenever possible, at least 30 days prior to your travel date in order to obtain the best airfare and registration rates. Should you be requested to travel extensively on behalf of the Company, a travel advance can be obtained by submitting a check request to the Accounting Department one week prior to departure.

In order for Company-related expenses to be reimbursed, you must fill out an Expense Report form. In order to comply with Internal Revenue Service regulations, the Expense Report must show and substantiate the following information:

1. The amount spent;
2. Date(s) of departure and return for each trip;
3. Business purpose of travel, meal and/or entertainment;
4. Date(s) and place(s) of travel, meal and/or entertainment;
5. Business relationship with the person entertained; and
6. Topic of discussion at business meal/entertainment.

The following are considered reasonable expenses which are reimbursable by the Company. Any expenses in excess of reasonable expenses or expenses greater than $25.00, without a receipt, will not be reimbursed by the Company.

1. Airfare will be reimbursed at economy/coach rates and should be booked through the Company travel agent, Rendezvous Travel, phone number (619) 454-3181, whenever possible.

2. Employees should rent subcompact or compact automobiles, when necessary. Often taxi cabs can be less expensive than a rental car during short trips. Only collision/damage insurance should be purchased from the rental agency. Nanogen has a $1,000,000 liability policy in place for hired and non-owned automobiles.

3. Employees should utilize moderately priced, single room hotel accommodations, such as Holiday Inn, Marriott, etc. The employee will be reimbursed for one call home per day and business calls only. Rental movies will not be reimbursed.

4. While traveling, the employee will be reimbursed for meals up to:

   Breakfast - $10.00
   Lunch - $15.00
   Dinner - $26.00

If a candidate or business associate is taken out for a meal, reimbursement will be limited to $20.00 per person for lunch and $35.00 per person for dinner.

Meals or travel for spouses of employees/candidates/business associates will not be reimbursed without prior approval from the Company President.

Any contemplated business gift or "goodwill" business meal must be preapproved by the Company President. The Internal Revenue Service allows a maximum deduction of $26.00 per person during a year for business gifts. No deduction is allowed for "goodwill" business meals.

Hotel bills must be detailed on your Expense Report to show the breakout of room charges, meals, telephone charges, etc.

When business meals or activities are attended by two or more Company employees, the highest ranking employee should list the expense on his or her Expense Report.

Approved Expense Reports must be submitted within five working days of trip return or date of expenditure.

Receipts for all expenses must be attached (if no receipt is available, the expense must be otherwise fully documented). Cash register receipts or credit card slips are required. Tear-tab receipts from restaurants are not acceptable. Company policy requires receipts for all expenditures of $25.00 or more, except mileage. Submit the Expense Report to your department supervisor for approval.

WORK SAFETY AND ACCIDENT REPORTING

Office personnel should follow standard work safety procedures and report any accident **immediately** to your supervisor. See the topic "First Aid" regarding First Aid Kits.

Laboratory personnel should follow standard laboratory safety procedures. If you have a question on any procedure, ask your supervisor prior to proceeding with the corresponding activity.

If there is an accident, injury or hazardous condition, follow these steps:

1. Take necessary steps to administer first aid to any injuries resulting from the accident/hazard. See "First Aid" regarding First Aid Kits.

2. Notify co-workers of the accident/hazard.

3. If necessary, bring the hazard under control or clean up any materials which have broken or spilled.

4. Immediately notify your supervisor of the incident.

Seek medical aid, if required, at a hospital emergency room. If there is any question as to whether a particular situation requires professional medical aid, resolve the question in favor of immediately obtaining professional medical aid. The nearest hospital emergency room is:

Scripps Memorial Hospital
9888 Genesee Avenue
La Jolla, CA 92037

Phone: (619) 457-6150

As soon as possible (within 24 hours) after you have been treated and are able, report the accident, injury or hazardous condition on the Nanogen "Unusual Occurrence Report." The report forms may be obtained from the Human Resources Department or the Receptionist.

EMERGENCY EVACUATION

Nanogen is committed to provide a safe environment for all employees and visitors. This is primarily a management responsibility, but is also an employee responsibility to ensure that safe work practices are followed and that hazards are identified and corrected. This includes all Company safety procedures and applicable safety and health regulations from various regulatory agencies as they relate to handling equipment, chemicals, radioisotopes and biologically hazardous materials.

Employees are expected to exercise sound judgment in performing their duties, to report immediately any accident or injury, unsafe condition or faulty equipment to their supervisor and to comply with all general and laboratory safety rules. Employees are responsible for reading and applying the procedures and precautions stated in laboratory safety training and procedure manuals located in each laboratory. Laboratory safety handbooks will be given to each employee on their first day of employment. If you have misplaced your handbooks or need another copy, please contact the Human Resources Department.

When an on-the-job injury or accident occurs, the employee must complete an Unusual Occurrence Report. Refer to "Accident Reporting" in this manual for additional information.

First aid supplies, fire blankets and fire extinguishers are located in designated areas of the Company's facilities.

FOR MEDICAL EMERGENCIES DURING WORKING HOURS -

1. Dial "0" and provide the Receptionist with the following information:
   - State that this is an emergency.
   - Identify the location of the victim/emergency.
   - Identify the nature of the emergency.

2. The receptionist shall immediately notify the following:
   - The appropriate fire/paramedic emergency services.
   - The Radiation Safety Officer (when appropriate).
   - The Human Resources Manager or Representative.
   - The appropriate department Vice President.
   - The Company President.

FOR MEDICAL EMERGENCIES AFTER WORKING HOURS:

- Dial "911" and provide the following information:
- State that this is an emergency.
- Identify the location of the victim/emergency.
- Identify the nature of the emergency.

FOR FIRE EMERGENCIES DURING WORKING HOURS:

1. Dial "0" and provide the receptionist with the location and nature of the emergency. If safe to do so, attempt to put out the fire. If not, evacuate the area immediately, ensuring that all persons are out of the building.

2. The receptionist shall immediately notify the following:
   - The Fire Department
   - The Facilities Landlord
   - The Human Resources Manager or Representative
   - The Company President

3. If the building is to be evacuated, the Receptionist shall make the appropriate announcement over the public address system.

FOR FIRE EMERGENCIES AFTER WORKING HOURS:

1. Dial "911" and provide the location and nature of the emergency.

2. All employees shall become familiar with the location of all emergency exits, emergency showers, eye wash stations and fire extinguishers and blankets. All emergency exits will be posted in the building.

SMOKING

In compliance with San Diego City Ordinance #0-17633, smoking shall not be allowed inside the building at any time.

GOOD LUCK!

Thank you for becoming a part of the Nanogen team. We are happy to have you with us. With your support, our Company will grow and prosper and continue to provide meaningful and challenging employment for you and other members of our community.

Nanogen INCORPORATED

ACKNOWLEDGMENT OF RECEIPT AND UNDERSTANDING

of the

EMPLOYEE HANDBOOK

I acknowledge receipt of Nanogen Incorporated's Employee Handbook. I have read this Handbook in its entirety and I acknowledge my responsibility to follow all company practices and procedures.

Employee: _____ JULY 1, 1994
Signature Date

DONALD D. MONTGOMERY
Please print

Received by: _____ 7/1/94
Human Resources Date

AO 120 (3/85)

TO: **Commissioner of Patents and Trademarks Washington, D.C. 20231**	**REPORT ON THE FILING OR DETERMINATION OF AN ACTION REGARDING A PATENT**

In compliance with the Act of July 19, 1952 (66 Stat. 814; 35 U.S.C. 290) you are hereby advised that a court action has been filed on the following patent(s) in the U.S. District Court:

DOCKET NO.	DATE FILED	U.S. DISTRICT COURT
00cv2369JM(RBB)	11/28/00	United States District Court, Southern District of California
PLAINTIFF Nanogen, Inc.		DEFENDANT Donald D. Montgomery; Combimatrix Corp.

PATENT NO.	DATE OF PATENT	PATENTEE
1 6,093,302	07/25/2000	Donald D. Montgomery; Combimatrix Corp.
2		
3		
4		
5		

In the above-entitled case, the following patent(s) have been included:

DATE INCLUDED	INCLUDED BY ☐ Amendment ☐ Answer ☐ Cross Bill ☐ Other Pleading	
PATENT NO.	**DATE OF PATENT**	**PATENTEE**
1		
2		
3		
4		
5		

In the above-entitled case, the following decision has been rendered or judgment issued:

DECISION/JUDGMENT		
CLERK	(BY) DEPUTY CLERK	DATE

Copy 1 - Upon initiation of action, mail this copy to Commissioner Copy 3 - Upon termination of action, mail this copy to Commissioner

Copy 2 - Upon filing document adding patent(s), mail this copy to Commissioner Copy 4 - Case file copy

JS 44
(Rev. 3/99)

CIVIL COVER SHEET

The JS-44 civil cover sheet and the information contained herein neither replace nor supplement the filing and service of pleadings or other papers as required by law, except as provided by local rules of court. This form, approved by the Judicial Conference of the United States in September 1974, is required for the use of the Clerk of Court for the purpose of initiating the civil docket sheet. (SEE INSTRUCTIONS ON THE REVERSE OF THE FORM)

FILED
00 NOV 28 PM 4:32
CLERK US DISTRICT COURT
SOUTHERN DISTRICT OF CALIFORNIA
BY: DEPUTY

I. (a) PLAINTIFFS

Nanogen, Inc., a Delaware Corporation,

DEFENDANTS

Donald D. Montgomery, an individual, and Combimatrix Corp., a Delaware corporation, and California corporation,

(b) COUNTY OF RESIDENCE OF FIRST LISTED PLAINTIFF San Diego
(EXCEPT IN U.S. PLAINTIFF CASES)

COUNTY OF RESIDENCE OF FIRST LISTED DEFENDANT King
(IN U.S. PLAINTIFF CASES ONLY)
NOTE: IN LAND CONDEMNATION CASES, USE THE LOCATION OF THE TRACT OF LAND INVOLVED.

(c) ATTORNEYS (FIRM NAME, ADDRESS, AND TELEPHONE NUMBER)
Brobeck Phleger & Harrison LLP
12390 El Camino Real
San Diego, CA 92130
858.720.2500

ATTORNEYS (IF KNOWN)

00 CV 2369 JM (RBB)

II. BASIS OF JURISDICTION (PLACE AN "X" IN ONE BOX ONLY)

- ☐ 1 U.S. Government Plaintiff
- ☒ 3 Federal Question (U.S. Government Not a Party)
- ☐ 2 U.S. Government Defendant
- ☐ 4 Diversity (Indicate Citizenship of Parties in Item III)

III. CITIZENSHIP OF PRINCIPAL PARTIES (PLACE AN "X" IN ONE BOX FOR PLAINTIFF AND ONE BOX FOR DEFENDANT)
(For Diversity Cases Only)

	PTF	DEF		PTF	DEF
Citizen of This State	☐ 1	☐ 1	Incorporated or Principal Place of Business In This State	☐ 4	☐ 4
Citizen of Another State	☐ 2	☐ 2	Incorporated and Principal Place of Business In Another State	☐ 5	☐ 5
Citizen or Subject of a Foreign Country	☐ 3	☐ 3	Foreign Nation	☐ 6	☐ 6

IV. NATURE OF SUIT (PLACE AN "X" IN ONE BOX ONLY)

CONTRACT
- ☐ 110 Insurance
- ☐ 120 Marine
- ☐ 130 Miller Act
- ☐ 140 Negotiable Instrument
- ☐ 150 Recovery of Overpayment & Enforcement of Judgment
- ☐ 151 Medicare Act
- ☐ 152 Recovery of Defaulted Student Loans (Excl. Veterans)
- ☐ 153 Recovery of Overpayment of Veteran's Benefits
- ☐ 160 Stockholders' Suits
- ☐ 190 Other Contract
- ☐ 195 Contract Product Liability

REAL PROPERTY
- ☐ 210 Land Condemnation
- ☐ 220 Foreclosure
- ☐ 230 Rent Lease & Ejectment
- ☐ 240 Torts to Land
- ☐ 245 Tort Product Liability
- ☐ 290 All Other Real Property

TORTS

PERSONAL INJURY
- ☐ 310 Airplane
- ☐ 315 Airplane Product Liability
- ☐ 320 Assault, Libel & Slander
- ☐ 330 Federal Employers Liability
- ☐ 340 Marine
- ☐ 345 Marine Product Liability
- ☐ 350 Motor Vehicle
- ☐ 355 Motor Vehicle Product Liability
- ☐ 360 Other Personal Injury

PERSONAL INJURY
- ☐ 362 Personal Injury - Med. Malpractice
- ☐ 365 Personal Injury - Product Liability
- ☐ 368 Asbestos Personal Injury Product Liability

PERSONAL PROPERTY
- ☐ 370 Other Fraud
- ☐ 371 Truth in Lending
- ☐ 380 Other Personal Property Damage
- ☐ 385 Property Damage Product Liability

CIVIL RIGHTS
- ☐ 441 Voting
- ☐ 442 Employment
- ☐ 443 Housing/ Accommodations
- ☐ 444 Welfare
- ☐ 440 Other Civil Rights

PRISONER PETITIONS
- ☐ 510 Motions to Vacate Sentence

HABEAS CORPUS:
- ☐ 530 General
- ☐ 535 Death Penalty
- ☐ 540 Mandamus & Other
- ☐ 550 Civil Rights
- ☐ 555 Prison Condition

FORFEITURE/PENALTY
- ☐ 610 Agriculture
- ☐ 620 Other Food & Drug
- ☐ 625 Drug Related Seizure of Property 21 USC 881
- ☐ 630 Liquor Laws
- ☐ 640 R.R. & Truck
- ☐ 650 Airline Regs.
- ☐ 660 Occupational Safety/Health
- ☐ 690 Other

LABOR
- ☐ 710 Fair Labor Standards Act
- ☐ 720 Labor/Mgmt. Relations
- ☐ 730 Labor/Mgmt. Reporting & Disclosure Act
- ☐ 740 Railway Labor Act
- ☐ 790 Other Labor Litigation
- ☐ 791 Empl. Ret. Inc. Security Act

BANKRUPTCY
- ☐ 422 Appeal 28 USC 158
- ☐ 423 Withdrawal 28 USC 157

PROPERTY RIGHTS
- ☐ 820 Copyrights
- ☒ 830 Patent
- ☐ 840 Trademark

SOCIAL SECURITY
- ☐ 861 HIA (1395ff)
- ☐ 862 Black Lung (923)
- ☐ 863 DIWC/DIWW (405(g))
- ☐ 864 SSID Title XVI
- ☐ 865 RSI (405(g))

FEDERAL TAX SUITS
- ☐ 870 Taxes (U.S. Plaintiff or Defendant)
- ☐ 871 IRS - Third Party 26 USC 7609

OTHER STATUTES
- ☐ 400 State Reapportionment
- ☐ 410 Antitrust
- ☐ 430 Banks and Banking
- ☐ 450 Commerce/ICC Rates/etc.
- ☐ 460 Deportation
- ☐ 470 Racketeer Influenced and Corrupt Organizations
- ☐ 810 Selective Service
- ☐ 850 Securities/Commodities/ Exchange
- ☐ 875 Customer Challenge 12 USC 3410
- ☐ 891 Agricultural Acts
- ☐ 892 Economic Stabilization Act
- ☐ 893 Environmental Matters
- ☐ 894 Energy Allocation Act
- ☐ 895 Freedom of Information Act
- ☐ 900 Appeal of Fee Determination Under Equal Access to Justice
- ☐ 950 Constitutionality of State Statutes
- ☐ 890 Other Statutory Actions

V. ORIGIN (PLACE AN "X" IN ONE BOX ONLY)

☒ 1 Original Proceeding ☐ 2 Removed from State Court ☐ 3 Remanded from Appellate Court ☐ 4 Reinstated or Reopened ☐ 5 Transferred from another district (specify) ☐ 6 Multidistrict Litigation ☐ 7 Appeal to District Judge from Magistrate Judgment

VI. CAUSE OF ACTION (CITE THE U.S. CIVIL STATUTE UNDER WHICH YOU ARE FILING AND WRITE A BRIEF STATEMENT OF CAUSE. DO NOT CITE JURISDICTIONAL STATUTES UNLESS DIVERSITY.)

[redacted] 256 Correction of inventorship of U.S. Patent No. 6,093,502. 28 USC SS 2201 & 2202, declaratory ruling of inventorship, & multiple state and common law claims.

VII. REQUESTED IN COMPLAINT:

☐ CHECK IF THIS IS A CLASS ACTION UNDER F.R.C.P. 23

DEMAND $ (BB) 35:0271

CHECK YES only if demanded in complaint:
JURY DEMAND: ☒ YES ☐ NO

VIII. RELATED CASE(S) IF ANY (See instructions):

JUDGE Jeff Bruiz DOCKET NUMBER

DATE November 28, 2000 SIGNATURE OF ATTORNEY OF RECORD Jeffery W. Guise

FOR OFFICE USE ONLY
RECEIPT # 26624 AMOUNT $150.00 APPLYING IFP JUDGE MAG. JUDGE

FDJS44